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INCREASED SENSITIVITY IN PHOTOMETRIC DETERMINATIONS

A THESIS

Presented to

The Faculty of the Division of Graduate

Studies and Research

by .

Robert Lawrence Barnes

In Partial Fulfillment

of the Requirements for the Degree


Doctor of Philosophy

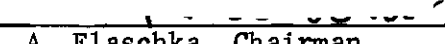
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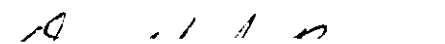
June, 1972

INCREASED SENSITIVITY IN PHOTOMETRIC DETERMINATIONS

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## SUMMARY

Three approaches for increasing the sensitivity of photometric determinations are explored. These approaches are based on increasing the magnitude of the parameters: absorptivity,  $a$ , effective path length,  $b$ , and effective concentration,  $c$ , of Lambert-Beer's Law,  $A = abc$ . Each of the approaches has provided increased sensitivity and it is felt that the techniques developed or combinations of them can be successfully applied to practical analysis when determining lower concentrations or using smaller samples or both.

### Solid and Homogeneous Extractions

Improved extraction techniques which provide increased enrichment and facilitate mixing and phase withdrawal are described. In the "solid extraction" technique organic compounds melting at slightly elevated temperatures are employed as solvents. The extraction is performed by heating the solution to be extracted with the solid solvent until the solvent is liquified, shaking the mixture to obtain liquid-liquid extraction, and then cooling to solidify the solvents and thereby facilitate phase withdrawal. In a more sophisticated technique, a "solid-homogeneous extraction" is obtained by employing a similar low-melting compound which, in addition, exhibits a highly increased solubility in water at elevated temperatures. In this case, upon heating the compound melts and then dissolves. Cooling results in homogeneous liquid-liquid extraction followed by solvent solidification. This latter technique facilitates mixing as

well as phase withdrawal. The advantages of these techniques for micro extractions and enrichment extractions prior to photometric measurement are illustrated and discussed.

Procedures for the application of the techniques are given for the copper (II) - PAN system. This specific system was selected because of its widespread use and familiarity.

#### Application of Sulfolane for Increased Sensitivity in Photometric Determinations

Sulfolane (tetrahydrothiophene -1,1-dioxide) was investigated as a color enhancing agent to increase the adsorptivity of various metal complexes. This work was centered around enhancement of the color of the cobalt thiocyanate complex. For this complex, sulfolane caused greater enhancement than any enhancing solvent previously reported. Attempts to apply this reagent to other thiocyanate complexes and to metal chelates with high absorptivity are discussed.

#### Design and Construction of a Spectrophotometer Incorporating Long-Path Microcells

The design and construction of a simple, single beam spectrophotometer capable of incorporating long-path microcells are described. Long-path microcells, 40 cm in actual length requiring 0.2 and 0.8 ml of sample for complete filling are employed. The divergence of the light beam entering the cell causes internal reflections within the cell. Accordingly, the effective path length is greater and in this case was found to be approximately 48 cm.

Operational procedures, preliminary tests, and examples of application of the constructed spectrophotometer are given.

## CHAPTER I

### THE PHILOSOPHY OF INCREASED SENSITIVITY IN PHOTOMETRIC DETERMINATIONS

#### Application of Photometric Methods

Since earliest civilization man has used the color of solutions to judge their quality. In this sense, colorimetry, the mother of modern spectrophotometry, must be ranked with the oldest of analytical methods. A survey of the present day's laboratories indicates that photometric methods comprise the largest portion of all analytical instrumental techniques.

The wide usage of photometers and spectrophotometers can be readily understood if the broad application of the instruments is considered. No other instruments can be applied so inclusively to analyses of organic and inorganic materials; cations, anions, and neutral species; and major, minor, and trace constituents. Photometric methods also allow differentiation between oxidation states and enable determination of suspended or particulate matter. The wide acceptance of photometry can additionally be attributed to generally high sensitivity, possibility of low-cost instrumentation, and simplicity of operation.

The large number of substances that can be determined by spectrophotometry provides a hint to the method's Achilles' heel. Since most spectrophotometric reagents are not very selective, there is a high probability of interference unless appropriate measures are taken. Frequently, isolation of the substance of interest is required. Such a separation step

is rarely easy and, with usual practice in mind, often virtually impossible. Once separation is achieved, however, the spectrophotometric determination usually becomes a simple task.

### Requirements of Photometric Methods

The primary demands on modern analytical chemistry can be summarized as<sup>1</sup> detecting and determining smaller amounts and obtaining results faster and more automatically. The most important requirements for photometric methods in satisfying these demands are sensitivity and selectivity. If these preliminary requirements are fulfilled, attention is directed to the secondary requirements of speed, simplicity of work, color stability, ruggedness, and adherence to Beer's Law.

Perhaps the best realization of these qualities has resulted from the application of classical, wet methods providing separation and enrichment, with a photometric finish. In particular, extraction-photometric determinations have been quite successful in aiding the resolution of many complex analytical problems.

### The Meaning of Sensitivity

The primary field of application for photometry is, by nature and tradition, determination of small amounts which are usually present in low concentrations. Consequently, sensitivity is of utmost importance. Unfortunately, the term sensitivity is used in modern analytical practice in an ambiguous manner. With strict regard to instrumental methods, the sensitivity is the slope of the calibration curve. As related to photometric determinations, the sensitivity is  $dA/dC$ , that is, the slope of the calibration curve in the usual absorbance versus concentration plot.

In other words, the smaller the change in concentration required to cause a certain change in absorbance, the higher the sensitivity of the particular method. In qualitative analysis the term sensitivity has been used for decades in a manner such that a method was said to be sensitive when a very small amount of substance could be detected. In this sense, sensitivity is closely related although not identical with the "limit of detection" or "limit of determination." In order to avoid confusion and still maintain the entrenched word, it is proposed that sensitivity be used with modifiers; basic sensitivity when relating to limit of determination, and slope sensitivity when relating to the instrument. The implications will become readily understandable from a graphical presentation. Consider the situation presented by Figure 1. The curve is steep at the beginning and the slope sensitivity high. As the concentration increases the curve levels off and this sensitivity decreases. The high slope sensitivity at the left-hand portion of the curve does not necessarily mean that very small amounts of sought-for substance can be determined. The lower limit of concentration at which one can operate depends on the signal to noise ratio. Suppose the dotted line is the limit at which operation is possible. Here the magnitude of the signal is at least three times the standard deviation of the noise as the criterion is commonly taken.<sup>2</sup> Thus, due to this limitation in basic sensitivity, operation is impossible within the range that would seem to offer optimal conditions when viewed solely with the slope sensitivity in mind.

The term basic sensitivity now seems quite appropriate, as it relates to the sensitivity existing at or near the base line of the calibration curve. Often the word sensitivity is used in a way including both

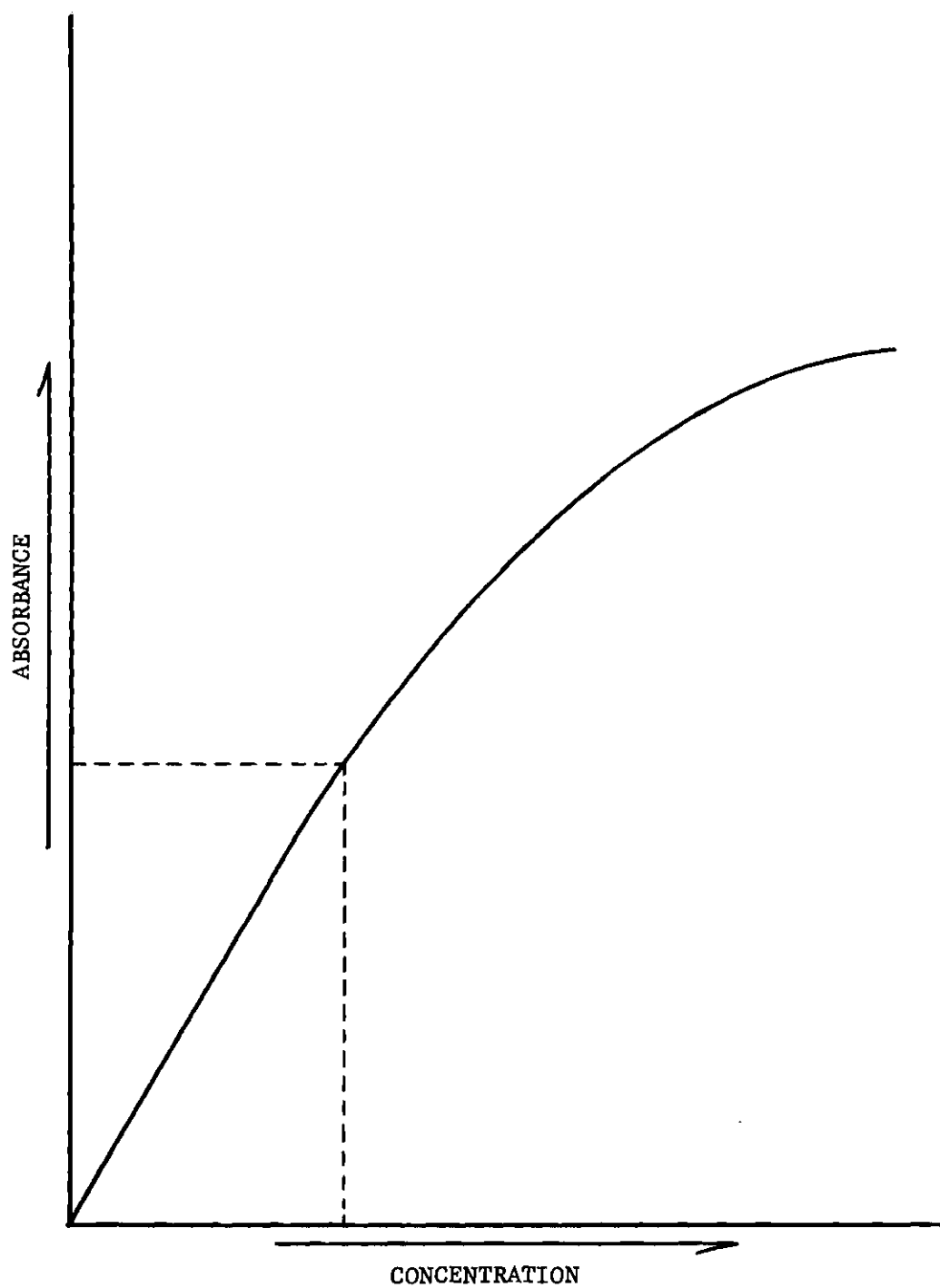


Figure 1. Illustration of Basic and Slope Sensitivities



special meanings, when it is desired to express that a method is generally a good one. When, from the context it can be seen which sensitivity is implied, the word may be used without a modifier as will be done in the course of later discussion. The basic sensitivity is frequently expressed in micrograms per square centimeter according to a proposal by Sandell.<sup>3</sup> If the assumption is made that the average spectrophotometer can detect an absorbance difference of 0.001 with certainty, a basis for the expression of the basic sensitivity of photometric methods may be established. This absorbance value corresponds to approximately 0.2 percent transmittance, usually readily attainable even with rather simple instruments.

From the preceding discussions it can be understood how a "more sensitive" method may, in fact, have a less impressive limit of determination than a "less sensitive" one due to a low signal to noise ratio limiting the "more sensitive" method at low concentrations.

#### Means of Increasing Sensitivity in Photometric Determinations

The possibilities existing for increasing sensitivity can be recognized upon examination of Lambert - Beer's Law.

$$A = abc \quad (1)$$

As pointed out in the discussion of the concept of sensitivity, the important principle of measurement is to have a high absorbance value for a low effective concentration of the species to be determined.

From a purely instrumental point of view, the better the signal to noise ratio, the smaller the concentration that can be determined with a certain reliability. Improving this ratio will consequently increase the

basic sensitivity. However, to increase the signal to noise ratio beyond levels presently attainable would require new optical and electronic components and new instrumental design features. In these respects the existing possibilities have almost been fully exhausted, at least within the limits of financial practicality. When working for improvement it is therefore better to concentrate on the parameters  $a$ ,  $b$ , and  $c$  of Lambert - Beer's Law.

#### Increasing the Effective Concentration, $c$

Effective concentration is defined as the concentration of that species which is subjected to the photometric measurement when the final solution is placed in the photometric cell. This species may be the sought-for species or contain it in a complex, adduct, etc. It may, however, be a completely different species. For example, one may determine dichromate by oxidizing an appropriate organic substance with it. The oxidation product (in an amount equivalent to that of the dichromate) may be extracted and the extract subjected to photometric measurement. From the effective concentration of that oxidation product in the extract, the conclusion is drawn as to the amount of dichromate present in the sample.

The effective concentration can usually be increased by an enrichment. To achieve this, extraction is one of the most applicable techniques. While enrichment extractions have been extensively employed, few deliberate attempts have been made to exhaust their potential. Problems of inefficient extraction and difficulty in recovering small volumes of extract have limited the enrichment. With small or micro samples enrichment extractions become exceedingly difficult. Methods developed in this

laboratory for facilitating extraction by a homogeneous technique and increasing the efficiency of phase withdrawal by solidification of the solvent are described in Chapter III and discussed elsewhere.<sup>4</sup>

#### Increasing the Absorptivity, $a$

Higher absorbance values would result from increasing the absorptivity,  $a$ . This method of attack generally implies synthesizing of new reagents. Such an approach has frequently brought success, but because of limited knowledge of exactly what structure is required for what purpose, the development of such improved reagents is time consuming and definite success is not assured. The theoretical maximum value of the molar absorptivity has been shown by Braude<sup>5</sup> to be of the order of 100,000 liters per mole-centimeter. Common values for the molar absorptivity of the species employed in the most sensitive photometric methods lie in the range of 20,000 to 40,000. Thus, no tremendous sensitivity increases can be expected from newly synthesized reagents. In addition, absorptivity is not the only parameter to be considered. The problems of selectivity, kinetics, availability, stability, and freedom from interferences must also be taken into account.

Another approach to increasing the absorptivity is to develop a means of enhancing the existing absorptivity of compounds or complexes already used for photometric determinations. An enhancing technique employing sulfolane has been tested with moderate success on the cobalt thiocyanate complex and is described in Chapter IV.

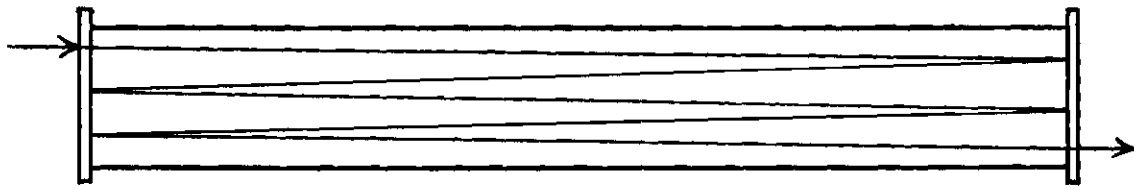
#### Increasing the Path Length, $b$

Finally, the length of the light path,  $b$ , remains for discussion.

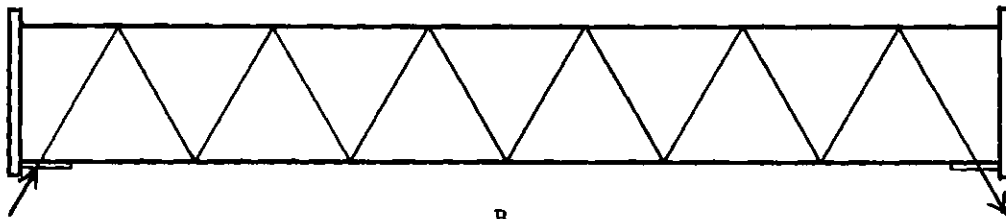
Obviously increasing the path length brings about an increase in absorbance. The most common path length for a spectrophotometric cell is 1 cm. Cells with 5 or 10 cm path lengths are readily available, but not every commercial instrument will accommodate them. In addition, such cells pay for the increased path length with a usually more than proportional increase in the volume of liquid required to fill the cell. Such a volume increase is, of course, undesirable and fully detrimental to any efforts made for enrichment prior to the photometric finish. In order to keep the volume low, use of a microcell would seem to be the logical step. What is commonly understood as a microcell, however, is a cell that is filled with very small amounts of liquid, but has a path length of millimeters or fractions of a millimeter and is thus, of course, unsuitable for the low absorbance of the solutions under discussion.

With growing emphasis on analysis of low concentrations in micro samples, what is needed is a special cell providing a long light path but requiring only a small volume of sample solution for complete filling. Two principal ways exist to achieve this goal. One approach is to employ multiple reflections within the cell; the other is to physically expand the cell length, employing a capillary to keep the volume small. In an undergraduate research program, the author pursued several designs in effort to obtain a long path length through multiple reflections. Representations of three of the most notable designs are shown in Figure 2.

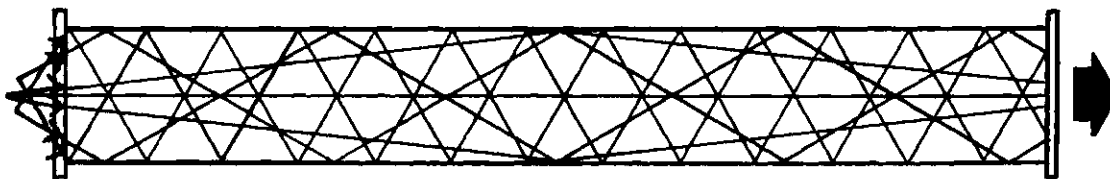
While this principle of increasing path length was illustrated, each of the designs shown is subject to a number of drawbacks and limitations.



A



B



C

Figure 2. Representations of Multiple Reflection  
Photometric Cells

Design A employs a glass tube with mirror ends to provide two or more internal reflections. Several such models tested gave effective path lengths of about 2.5 times the actual cell length. However, a relatively large cell size and, therefore, volume was required for mirror ends sufficient in size to provide adequate reflectance and thus attainment of a measurable light intensity.

Design B employs an internally polished stainless steel or slivered glass tube. Alternatively, a square or rectangular cell with two opposite mirror walls could be used. In addition, the mirrors could be set at angles to form a trapezoid. For reasonable detector response, a highly collimated light beam is required for all constructions of this type design. Such collimation is very difficult to obtain without elaborate and expensive optics. Further, without at least one transparent side, the cell contents cannot be effectively examined for air bubbles and particulate matter, and the square, rectangular, or trapezoidal models are exceedingly difficult to construct as a true microcell.

Design C utilizes a highly dispersing light beam. Without slivered walls (which would again make examination of the contents impossible) very little of the entering light ever reaches the detector.

All of the designs described are subject to high losses of incident light, a factor leading to difficulties in procuring a sufficiently intense and stable light source and often requiring extensive signal amplification. Designs A and B are also difficult to properly align within the light beam.

This previous experience with multiple reflection cells led to

concentration on the other, and simpler approach to building a long path microcell. A mere physical expansion of the cell length using a capillary for the body, can fulfill the requirements. This capillary cell and the method of its mounting and alignment (see Chapter VI) seems to provide the best combination of simplicity and performance.

#### Technique Combinations

While each of the techniques discussed for increasing the sensitivity in photometric determinations may, in itself, perform well within a reasonable range, it is obvious that highest sensitivity can be obtained from combinations of these techniques. In particular, the micro extraction, providing increased enrichment, can be readily coupled with the long path microcell. If the developed extraction technique can provide three to four times greater enrichment than conventional techniques (Chapter III) and the microcell has an effective light path of 50 cm, increases in sensitivity by a factor of 150 to 200 over conventional determinations can be expected.

## CHAPTER II

### EQUIPMENT AND CHEMICALS

#### Laboratory Equipment

##### Spectrophotometers

The spectrophotometric measurements in the experiments described in Chapter III were carried out on a Bausch and Lomb Spectronic 20 modified in the following way to permit use of 1.5 ml samples. The cell compartment was adjusted to raise the sample tube so that the light beam passes through the tube as close to the bottom as possible but without striking the spherical portion. The slit was masked with black cardboard to cut off the upper half of the light beam.

A Cary 16 Spectrophotometer was employed for the absorbance measurements in Chapter IV and in the measurement of the effective path length of the long-path microcell (Chapter VII).

The long-path spectrophotometer described in Chapters V, VI, and VII was employed exclusively in the examples given in Chapter VIII.

The absorbance curves were obtained with a Bausch and Lomb Spectronic 505 Spectrophotometer.

The atomic absorption measurements (Chapter IV) were performed on a Jarrell Ash 82-270 Atomsorb Atomic Absorption Spectrophotometer.

##### pH Meter

All required pH measurements were made with a Corning Model 7 pH meter. This instrument was calibrated with a Leeds & Northrup Company



6.86 buffer.

#### Microburet

A Coleman Microtrator Model 6-821 microburet with a precision of approximately  $\pm 0.02 \mu\text{l}$  was employed for the photometric titration discussed in Chapter VIII.

#### Glassware

Common laboratory glassware such as beakers and flasks were employed as needed. Micro glassware including 1 to 5 ml beakers, flasks and volumetric flasks were used extensively in the "solid and homogeneous" extraction techniques. All volumetric glassware was Class A and was used without additional calibration.

#### Chemicals

Distilled water, passed through a mixed-bed deionizer column was used exclusively. All common acid, base, and buffer solutions were prepared from the reagent grade chemicals. Metal salt solutions were prepared from J. T. Baker "Analyzed Reagent" grade salts.

All organic chemicals and solvents were reagent grade or better with the exceptions of sulfolane and benzophenone. The sulfolane used in the initial experiments was obtained by collecting the fraction from  $121^{\circ}$  to  $125^{\circ}\text{C}$  when distilling Eastman Organic Chemicals practical grade at 1.9 mm of mercury. Sulfolane used in the quantitative comparison experiments was specially distilled and provided by J. T. Baker Chemical Company Research Division. The benzophenone employed was a purified grade melting at  $47^{\circ}$  to  $48^{\circ}\text{C}$  and showing no indication of trace metal contaminants when tested with PAN.

Solutions of PAN and other water insoluble reagents were prepared using 95 percent ethanol.

## CHAPTER III

### HOMOGENEOUS AND SOLID EXTRACTIONS

#### Introduction

As discussed in Chapter I, solvent extraction followed by photometric measurement on the extract has proved to be highly successful in applied analysis. Extraction can provide separation from interfering species and from an unfavorable matrix. The extraction step can additionally be used to achieve an enrichment of the species to be subjected to photometric measurement and thus serve to increase the sensitivity.

Extraction - photometric methods are widely applied and of interest when the sought-for substances are present in micro amounts. The methods are used throughout the spectrum of applied analysis ranging from clinical, biochemical, and environmental analysis through product quality control to the characterization of advanced materials. In spite of the wide uses, possibilities for perfecting the extraction - photometric techniques have not been exhausted. In other chapters, means for increasing the overall sensitivity of the methods by improving the sensitivity of the photometric measurement are discussed. This chapter concentrates on increasing the overall sensitivity by improving the extraction process, via facilitating mixing and phase withdrawal, and thereby enabling attainment of a higher level of enrichment. Such improvement should allow application of extraction - photometric techniques to even lower amounts of constituents or smaller samples or both.

### Problems Associated with Extractions

In an enrichment extraction of traces of a species from a large volume of aqueous phase, the smaller the volume of water-immiscible organic solvent employed, the greater the enrichment achieved. In practice, however, when a very small volume of organic solvent is used, solvent droplets are formed that often remain dispersed in the aqueous phase, hang from the underside of the water surface, or cling tenaciously to the vessel walls. Transfer of these droplets for quantitative phase withdrawal is very difficult. Washing with additional solvent is necessary for complete removal of all of the extracted material, and therefore the enrichment is, at least in part, nullified. In some cases, enrichment can be improved after extraction by evaporating some of the solvent; however, such measures can result in losses, adverse reactions, or development of turbidity due to water. Dilution of the extract to a known volume is required in many procedures to allow quantification. This dilution can severely impair enrichment.

When extraction techniques are applied to small samples, that is, in microchemistry, the difficulties increase. Separatory funnels cannot be applied to the micro volumes and the siphoning employed in phase removal is inefficient and several rinsings with solvent are often required. In addition, the mixing of phases in micro extractions can be a tedious and difficult process. This is especially true considering the time required for complete equilibration of some systems. Although techniques have been developed to remedy some of the difficulties mentioned above, further improvements in the extraction step are needed, to result in an

extraction technique that is operative on the micro scale, allows ready phase removal and provides high enrichment.

### Solid Extraction

One possibility for the desired improvement in extraction technique is the use of what may be termed "solid extraction." In this technique, the organic phase, which is liquid during the actual extraction process, is solidified to facilitate its withdrawal from the aqueous phase. The approach is readily realized by employing as extracting solvent a suitable compound that is a solid at or slightly below room temperature. This approach was described as early as 1959 by Kuznetsov and Seryakova<sup>6</sup> who employed molten carbowaxes as extracting solvents. More recently, Fujinaga and coworkers<sup>7</sup> have reported the use of molten naphthalene to extract copper (II) and zinc as the oxinates. Gillet<sup>8</sup> extracted traces of chromium (III) into molten 8-hydroxyquinoline. In their discussion, all authors put great emphasis on the ease of phase withdrawal, but pay no attention to improving enrichment. This is understandable in light of the photometric finish to which the extracts were subjected. For the photometric measurement it is necessary to dissolve the separated bead, and using a photometer of conventional design, dilution to a rather large volume is required. Without this dilution it is impossible to fill the photometric cell to a level high enough to prevent splitting of the light beam by the surface of the liquid. Any enrichment originally achieved is decreased by the dilution or even lost. The long-path microcells discussed in Chapters V, VI, VII are especially applicable to this problem and have, indeed, been designed with these extraction techniques in mind.

Experiments performed in this laboratory<sup>4,9</sup> have clearly demonstrated the high enrichment derived from solid extraction. In this work, benzophenone, 2-methylnaphthalene, and 1-nitronaphthalene were found to exhibit the characteristics desired for solid-extraction solvents. Phase separation and phase withdrawal were excellent, and enrichment extractions of trace constituents from large volumes of aqueous phase into small amounts of solvent proved generally successful.

#### Homogeneous Extraction

When the volumes of the aqueous and organic phases are both small, the problem of mixing the two phases was, of course, in no way solved by the solid extraction technique. As previously mentioned the mixing process can be very tedious with certain systems where equilibration is slow. It was felt that if the two phases could be made temporarily, a single homogeneous phase, equilibration could be attained much easier and faster.

#### Consolute Temperatures

Certain organic compounds form with water, systems that exhibit both upper and lower consolute temperatures (or critical solution temperatures); that is, above the upper and below the lower consolute temperatures the compound and water are infinitely miscible. Between these two temperatures miscibility is limited and phase separation occurs. The German word for the range where immiscibility prevails is "Mischungslücke" which might be translated as mixing hole. This term becomes clear when inspecting a graphical presentation of the situation as shown in Figure 3<sup>10</sup> for the classical nicotine-water system. Such a system could be applied to extraction in the following manner. The appropriate organic solvent

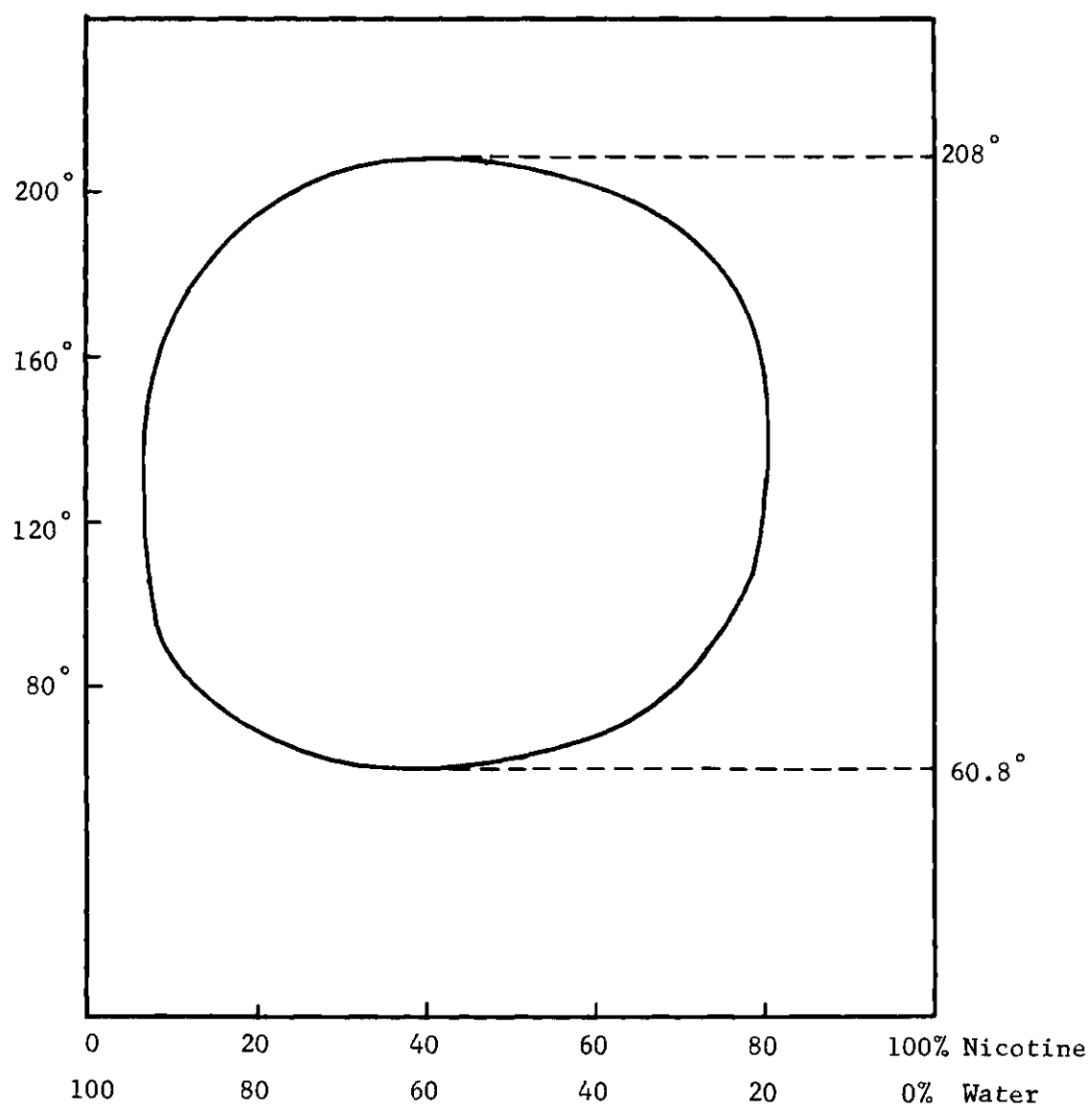


Figure 3. The Nicotine-water System

is added to the aqueous phase containing the species to be extracted. The system, showing two phases at room temperature, is then heated above the upper consolute or cooled below the lower consolute temperature and homogeneity is obtained. Respectively, cooling or warming the solution to room temperature could then effect "extraction from homogeneous solution" or simply "homogeneous extraction." These terms are coined in full analogy to those of "precipitation from homogeneous solution" and "homogeneous precipitation." Systems having a lower as well as an upper consolute temperature are especially interesting for possible application to extractions since they can either be cooled or heated to obtain homogeneity and thus offer greater flexibility. Table 1 lists a number of systems of this type.<sup>10</sup>

Table 1. Systems with Lower and Upper Consolute Temperatures

System	Lower Temp. C	Upper Temp. C
Nicotine-Water	60.8	208
Methyl ethyl ketone-Water	- 6	133
1-Methyl piperidine-Water	48	> 250
2-Methyl piperidine-Water	79	227
4-Methyl piperidine-Water	85	189
Diethylamine-Water	143	*
Triethylamine-Water	18.5	*
$\beta$ -Picoline-Water	49	153
$\gamma$ -Collidine-Water	6	225

\* For these systems no upper consolute temperature has been experimentally shown because it has not been possible to attain the requisite temperature. It is probable that these systems are fundamentally the same as those having two consolute points.



It is interesting to note that systems exhibiting lower and upper consolute temperatures are not limited to those having water as one of the components. For example, the Glycerol-m-Toluidine system has lower and upper consolute temperatures of  $6.7^{\circ}$  and  $120^{\circ}$ , respectively.

#### Preliminary Testing of Homogeneous Extracting Solvents

Of the systems listed in Table 1, methyl ethyl ketone-water seemed to have the greatest potential for the applications desired. Methyl ethyl ketone was tested as a solvent for the extraction of copper (II) - PAN as follows: to the aqueous copper solution in a small flask are added pH 5 acetate buffer and a few drops of ethanolic PAN solution. An approximately equal volume of methyl ethyl ketone is then added and the flask cooled in an ice-ethanol bath until homogeneity is achieved. The system is removed from the ice bath and warmed gently until room temperature is reached.

In this experiment, although a homogeneous solution was obtained and homogeneous extraction demonstrated in principle, a difficulty was encountered. When the system had reattained room temperature, enough ketone remained in the aqueous phase to cause incomplete extraction. The violet copper - PAN complex was present in both phases. The possibility was considered that the alcohol introduced when adding the PAN was responsible for this condition. Therefore, the experiment was repeated, this time adding a minute amount of solid PAN reagent directly to the ketone, rather than adding ethanolic PAN to the aqueous phase. The results were the same, proving that the alcohol is not responsible for the incompleteness of the extraction.

It was thought that systems having both lower and upper consolute temperatures might tend to exhibit high mutual solubility at all temperatures and thus be especially susceptible to the problem of incomplete extraction which was observed. Consequently, effort was directed toward finding substances which with water exhibit only an upper consolute temperature without having a lower consolute point. During this search a better approach was envisioned, namely, a combination of the solid and the homogeneous extraction techniques. The use of an organic compound that is highly soluble in water at elevated temperatures and that both separates and solidifies at room temperature could enable such an extraction. Compounds of this type would perhaps tend to offer less solubility at room temperature than those previously discussed.

While extractions utilizing this combination of techniques (discussed in the following section) were being successfully conducted, Murata and coworkers<sup>11</sup> did demonstrate an effective homogeneous liquid - liquid extraction method for one specific system. Propylene carbonate was used to extract iron (III) - thenoyltrifluoroacetate from aqueous solution. The propylene carbonate - water system has only an upper consolute temperature at 73°. Although the solubility of propylene carbonate in water was determined by the authors to be approximately 2 ml in 10 ml of water, at room temperature, complete extraction of the iron complex was reported. It is apparent that the most important criterion for efficient extraction is not the degree of mutual solubility of the organic and aqueous phases, but rather the solubility of the species being extracted in the organic phase (containing a small amount of water) versus its solubility in the

in the aqueous phase (containing a small amount of organic).

#### Solid - Homogeneous Extraction

From solubility tables<sup>12</sup> a number of compounds were selected as possible "solid-homogeneous solvents." A listing of these compounds with their solubilities and melting points is given in Table 2. After preliminary testing of these compounds, piperonal (3,4-OCH<sub>2</sub>OC<sub>6</sub>H<sub>3</sub>CHO) was selected as showing the most promise for extracting copper (II) - PAN and other metal chelates. Caffeine, interestingly displayed excellent solubility characteristics, but failed to extract panates or other chelates. Other substances tested as solid-homogeneous solvents, which upon cooling did not first separate as a liquid and then solidify, also failed to extract the metal complexes. This result is understandable since direct separation as a solid is essentially a process of recrystallization.

#### Use of a Mediating Solvent

Piperonal with slight solubility in water at room temperature, infinite solubility at 78°C, and a melting point of 37°C (see Table 2) performed well in the preliminary tests and seemed to fulfill the requirements as a solid-homogeneous solvent. However, when tested under actual extraction conditions with the aqueous phase approximately buffered and with PAN or another extractant dissolved in the piperonal, complete miscibility of the aqueous and organic phases could not be readily achieved. This observation is understandable when one considers that with addition of a foreign substance to the system, the mutual solubilities of the liquids additionally depend on the nature and quantity of the added material. If this material is soluble in only one of the two liquids, the mutual solubilities of

Table 2. Some Possible "Solid-Homogeneous Solvents"

Compound	Melting Point °C	Solubility (g/100 ml)	
Piperonal	37	0.2 cold	$\infty$ <sup>76*</sup>
Phenol	41	6.7 <sup>16</sup>	$\infty$ <sup>66</sup>
2,6-Dinitrophenol	61.8	very slightly soluble cold	very soluble hot
m-Nitrophenol	96	1.35 <sup>25</sup>	13.3 <sup>90</sup>
p-Nitrophenol	114	1.6 <sup>25</sup>	26.9 <sup>90</sup>
o-Phenylenediamine	102	4.15 <sup>35</sup>	733 <sup>81</sup>
p-Phenylenediamine	139	3.8 <sup>24</sup>	669 <sup>107</sup>
Phlorizin	108-9	0.1	very soluble hot
Protocatechuic Acid	199 d	1.82 <sup>14</sup>	27 <sup>80</sup>
Raffinose	118-9	14 <sup>20</sup>	$\infty$ hot
Succinic acid	185	6.8 <sup>20</sup>	121 <sup>100</sup>
2-Furan propanol	54	insoluble cold	soluble hot
Caffeine	235-7	1.35 <sup>16</sup>	45.5 <sup>65</sup>

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\*All superscript numbers indicate temperature.

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these liquids will be reduced. For example,<sup>10</sup> 0.1 mole of potassium chloride per liter of water raises the upper consolute temperature of the phenol-water system by about 8°; a similar concentration of naphthalene in the phenol raises the consolute temperature by about 20°. Therefore, it is to be expected that PAN or similar water insoluble extractants dissolved in piperonal, and acetate buffer, insoluble in the organic phase, dissolved in the water, should reduce the mutual solubility of the system.

To resolve these difficulties a volatile mediating solvent may be employed. The solvent, soluble in both phases, would increase the mutual solubility to a degree that complete homogeneity is attained. The volatile solvent, for example, acetone, could be readily removed by heating, thereby reestablishing two phases and in the course of this process, achieving homogeneous extraction.

### Procedures

The procedures given here are provided to illustrate how the solid and solid-homogeneous techniques can be used. With slight modifications, the techniques may be applied to other solvents and to a wide range of extractants. Specifics for application to other systems should be worked out for each particular case and experience from conventional extractions will almost always give reliable guide lines. The solubilities of extractants and their metal complexes may differ greatly between solid and conventional solvents and thus extraction may be complete in fewer steps, but the reverse might also be true. However, selectivity is not expected to be greatly changed because the ratio of the solubilities and thereby the partition coefficients should not be altered significantly.

Solid Enrichment Extraction of CU(II)-PAN into Benzophenone

1. Into a 200-ml Erlenmeyer flask with ground glass stopper place 100.0 ml of the roughly neutralized sample solution containing up to  $5 \times 10^{-5}$  millimoles ( $= 3 \mu\text{g}$ ) of copper. Add 10 ml of 0.1 F acetate buffer of pH 5, 0.50 ml of 0.010 F PAN solution, and 1.0 g of benzophenone.

2. Place on hot plate and warm until the benzophenone liquifies. Remove from the heat, stopper the flask, and shake vigorously for 2-3 minutes.

3. Remove the stopper and rinse it with some water. Add a few drops of 0.1 percent aqueous sterox SE surfactant solution and cool under tap water or in an ice bath. If supercooling persists initiate solidification by seeding with a few crystals of benzophenone.

4. Decant the liquid from the bead into another 200-ml Erlenmeyer flask with ground glass stopper. Add 0.50 ml of PAN solution and 1.0 g of benzophenone and repeat the extraction according to steps 2 and 3. Decant the aqueous phase from the bead and wash with some water.

5. Place the two beads into a 10-ml beaker. Take the beaker with the beads on a (preferably fast reading) balance and add 2.00 g of benzene or m-xylene. After dissolution is effected add a spatula tip of anhydrous sodium sulfate.

6. Decant the solution from the salt into the photometer tube and measure the absorbance against a reagent blank in a modified Spectronic 20 at 555 nm.

7. Read the result from the calibration curve obtained in the customary way by carrying solutions containing known amounts of copper through the procedure.

### Solid Micro Extraction of CU(II)-PAN into Benzophenone

1. Into a 1-ml Griffin beaker place 0.200 ml of the roughly neutralized sample solution containing up to  $2 \times 10^{-5}$  millimoles ( $= 1 \mu\text{g}$ ) of copper. Add 0.05 ml of 0.1 F acetate buffer pH 5, 0.05 ml of 0.010 F PAN solution, and 25 mg of benzophenone.
2. Place on a hot plate adjusted to a temperature sufficient to melt the benzophenone. Insert a micro stirrer and stir vigorously for 2-3 minutes.
3. Remove the stirrer, rinse with 1 drop of water and add one micro drop of surfactant solution. Remove the beaker from the hot plate and cool in an ice bath. If supercooling persists seed with a micro crystal of benzophenone.
4. Decant the aqueous phase into another 1-ml Griffin beaker and wash with 2-3 drops of water. Add 0.05 ml of PAN solution and 25 mg of benzophenone and repeat the extraction according to steps 2 and 3. Decant the aqueous phase from the bead and wash with some water.
5. Place the two beads into a 2-ml beaker. Tare the beaker with the beads on a (preferably fast reading) balance and add 1.4 g of benzene. After dissolution is effected add a spatula tip of anhydrous sodium sulfate.
6. Decant the solution from the salt into the photometer tube and measure the absorbance against a reagent blank in a modified Spectronic 20 at 555 nm.
7. Read the result from a calibration curve obtained in the customary way by carrying solutions containing known amounts of copper through the procedure.

Solid Homogeneous Extraction of CU(II)-PAN into Piperonal Using Acetone

1. Into a 4-ml Erlenmeyer flask place 2.00 ml of the roughly neutralized solution containing up to  $2 \times 10^{-5}$  millimoles ( $= 1 \mu\text{g}$ ) of copper. Add 0.2 ml of 0.1 F acetate buffer pH 5, 0.05 ml of 0.010 F PAN solution and 0.10 g of piperonal.
2. Place on a steam bath and when the piperonal has melted add acetone dropwise and with swirling until a homogeneous solution is obtained.
3. Let the flask remain on the steam bath to volatilize the acetone. Alternatively, remove the acetone by passing a stream of air or nitrogen through the solution using a narrow diameter glass tube drawn out to a fine tip.
4. When the acetone has been removed and the piperonal has separated add 2-3 drops of surfactant solution and cool under tap water or in an ice bath. If supercooling persists seed with a crystal of piperonal.
5. Decant the aqueous phase and wash with some water. Tare the flask with the bead on a (preferably fast reading) balance and add 2.00 g of m-xylene. After dissolution is effected add a spatula tip of anhydrous sodium sulfate.
6. Decant the solution from the salt into a photometer tube and measure the absorbance against a reagent blank in a modified Spectronic 20 at 555 nm.
7. Read the result from a calibration curve obtained in the customary way by carrying solutions containing known amounts of copper through the procedure.



### Results and Discussion

In all three procedures a limit is given for the amount of copper in the sample. This limit has been set very low in order to stay within the realm of trace analysis. Somewhat greater amounts can be present; there will be sufficient reserves of PAN and extracting solvent to achieve complete extraction. With much higher amounts, the extraction may not be complete and changes in the procedure may become necessary. With the limiting amounts as given, the absorbance readings on the modified Spectronic 20 are in the neighborhood of 0.4. The rather high readings for microgram amounts of copper show the benefit of keeping the final volume small. Still, in the case of the micro extraction, two beads of 25 mg each had to be dissolved to about 2 ml in order to provide adequate filling of the photometric cell. It is easy to imagine the magnitude of improvement when using the long-path microcells described in Chapters V, VI, VII.

It should be pointed out specifically that dissolving the solvent bead to a known total weight rather than to a specified total volume has been prescribed. The reason for this is that more flexibility is available and that adjustment to the smallest amount necessary to fill the tube is readily possible. With dilution to volume a volumetric flask is commonly employed which in most cases yields a volume far greater than needed.

As previously mentioned the solubility of piperonal may be greatly reduced under the actual extraction conditions. If limited solubility causes problems, the use of the mediating solvent should eliminate them.

When developing the procedures given, completeness of extraction

was tested by saving the decanted aqueous phases and subjecting them to exhaustive extraction with PAN into chloroform. The chloroform extracts were measured photometrically. Alternatively, three identical sample aliquots were extracted two, three, and four times according to the procedure and the absorbances compared. Two extractions into benzophenone removed over 99 percent of the copper present and are thus considered to be sufficient. Interestingly, in the homogeneous extraction procedure, one extraction suffices. To state that this is due to the application of homogeneous extraction would be a wrong conclusion since a different extracting solvent was employed. Homogeneous extraction is only in part responsible. The solvent itself probably has the more significant influence on the efficiency.

In solid extraction the separated organic phase sometimes does not coalesce even with the surfactant added. In such cases slight rewarming and cooling usually brings about the desired results.

The copper-PAN systems were selected, not because it seemed necessary to add one more method for copper to the already existing legion of procedure, but primarily because of the familiarity of the author with these systems. In addition, some PAN complexes exhibit a peculiar behavior. As was especially demonstrated by Flaschka and Weiss<sup>13</sup> and Zolotov and co-workers<sup>14</sup> for the extraction of the PAN complexes of cadmium and indium, respectively, the initially completely extracted metal complexes decompose in part and upon prolonged shaking, metal returns to the aqueous phase until an equilibrium is reached. When cadmium and indium PAN complexes were extracted using solid and solid-homogeneous techniques no return of

metal was encountered. Obviously, the metal remains "frozen" in the drop or bead of solvent.

## CHAPTER IV

EXPERIMENTS ON THE APPLICATION OF SULFOLANE  
FOR INCREASED PHOTOMETRIC SENSITIVITYApplication of Sulfolane as a Nonaqueous Solvent

Sulfolane (tetrahydrothiophene 1,1-dioxide, tetramethylenesulfone) has become of considerable interest as nonaqueous solvent in the past decade. This member of the class of relatively inert, polar solvents has comparatively recently been made available in commercial quantities. The extremely slight acid and base character of sulfolane makes it appropriate as a solvent in titrations of very weak acids and very weak bases; in addition, it is nonleveling for strong acids and strong bases. Sulfolane provides an extremely wide potential span of about 2000 mv, between solutions containing excess strong acid and excess strong base, making it an excellent solvent for differentiation of both mixtures of acids and mixtures of bases.<sup>15</sup> Sulfolane's high dielectric constant and relative overall inertness enable reactions to be carried out that are usually masked by either association reactions of the solute, in solvents of low dielectric constant, or by the solvent itself, in the case of water and other more reactive solvents. In addition, sulfolane is useful in the study of homoconjugated AHA- and BHB+ complexes because of its low tendency for hydrogen bonding.<sup>16</sup> Several of the physical properties of sulfolane have been reported: bp 285 °C, mp near 28 °C<sup>17</sup>; density 1.2623 g per ml, viscosity 0.1029 poise, and dielectric constant 43.3 (all at 30 °C).<sup>18</sup>

### Initial Investigations

Indications of the wide variety of substances soluble in sulfolane in the studies cited above and others<sup>19,20,21</sup> led to the initial interest in the solvent by this laboratory as a possible solvent for determinations involving chelates. It was specifically desired to find a solvent which would rearrange the order of metal-chelate stability constants. Such rearrangements of stabilities, if possible, could resolve a number of analytical problems commonly associated with determining metals in the presence of each other with chelating agents.

Preliminary investigations employing sulfolane in this light failed to provide the desired effects. Although sulfolane indeed proved to be a good solvent for a large number of inorganic and organic compounds, the chelate formers of most interest such as EDTA, EGTA [ethyleneglycol bis (2-aminoethyl ether) tetraacetic acid], and DCTA (1,2-diaminocyclohexane-tetraacetic acid) exhibited very poor solubility in the solvent. To obtain a solution ever  $10^{-3}$  formal in EDTA required addition of a relatively large amount of an amine such as N-methylbenzylamine which, in turn, hindered the evaluation of chelate stabilities. Most importantly, initial experiments with EDTA and other chelating agents indicated that the stabilities of the metal chelates are shifted to different values, but the order of stability is not altered.

During these investigations it was observed that, for a number of metal salts, the solution color in sulfolane was enhanced over the color exhibited in aqueous solution. Specifically, transition metal salts such as the nitrates of Cu(II), Ni(II), Cr(III), Co(II), and Fe(III) showed

significant increases in color intensity in sulfolane solution.

#### The Vogel Reaction

In 1875 H. W. Vogel<sup>22</sup> originated the test for cobalt ions which bears his name. The Vogel test consists of adding thiocyanate ions to the solution to be analyzed and then adding a suitable organic solvent. The so-called "Vogel Blue" indicates the presence of cobalt ions. Tomula<sup>23</sup> first proposed the photometric determination of cobalt by means of the blue thiocyanate complex. According to this method, a 50 percent by volume aqueous acetone solution containing 5 g of ammonium thiocyanate is employed to form the blue color. Young and Hall<sup>24</sup> used an amyl alcohol-water mixture to extract the complex from aqueous solutions acidified with hydrochloric acid. Uri<sup>25</sup> studied the stability of the complex in ethyl alcohol-water mixtures and optimised the conditions for a photometric determination.

Several theories have been postulated to explain the blue color, however, none is completely satisfactory. Since the pink color of aqueous cobalt returns upon dilution, Feigl<sup>26</sup> suggested that the blue is probably due to solvation of a complex cobalt thiocyanate such as  $K_2[Co(NCS)_4]$ .

Because of the widespread use of this reaction and its many modifications, and the different theories explaining the origin of the blue color, West and DeVries<sup>27</sup> studied the reaction in the presence of several organic solvents. The authors tried to relate the dielectric constant of the solvents commonly employed as enhancing agents or color developers\*

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\* Although the organic solvents employed has been referred to as "developer," it is felt that enhancing agent" is a better term, since in the presence of high concentrations of thiocyanate a weakly colored blue complex exists without organic solvent.

to the intensity of the blue color formed. In this study 1 ml of 0.02 F cobalt nitrate and 5 ml of 1 F potassium thiocyanate were added to a 10-ml volumetric flask. Enough of the organic solvent to be tested was added to make the final solution 4.28 F in it. The solutions were then diluted to the mark with distilled water and the photometric measurement made at 620 nm. The results are given in Table 3.

Table 3. A Comparison of Color Enhancing Agents

Enhancing Agent	Dielectric Constant	Absorbance
Methanol	32.6	0.015
Ethyl alcohol	25.0	0.090
n-Propyl alcohol	20.8	0.503
Isopropyl alcohol	15.7	0.785
sec-Butyl alcohol	15.5	0.920
tert-Butyl alcohol	3.76	1.16
Dioxane	2.1	0.885
Acetone	19.5	1.10

The conclusion was that while the dielectric constant appears to play a role in the formation of the Vogel Blue, other factors such as molecular size must also influence the color enhancing properties. The authors stated that  $[\text{Co}(\text{NCS})_6]^{-4}$  is formed in mixed solvents and that this ion is probably responsible for the formation of the blue color by its selective attraction of organic molecules resulting in a dehydration of the complex

cobalt ion. Acetone and tert-butyl alcohol were suggested as the best enhancing agents with the latter being preferred because of its low vapor pressure.

#### Sulfolane as a Vogel Blue Enhancing Agent

The enhancement characteristic which sulfolane exhibited for metal salt solutions led to speculation as to its value in connection with the Vogel reaction. Accordingly, a comparison was made with several of the better color enhancing agents reported in Table 3. The experiments were performed under conditions identical to those of West and DeVries; the results are given in Table 4 with the absorbance values being measured on a Cary 16 Spectrophotometer at 620 nm.

Table 4. A Comparison of Sulfolane with Other Vogel Blue Enhancing Agents

Enhancing Agent	Ml. 0.02 $\text{F Co}^{+2}$ Added	Absorbance
Sulfolane	1.00	2.80
tert-Butyl alcohol	1.00	1.09
Acetone	1.00	1.26
Dioxane	1.00	0.820
Sulfolane	0.50	1.42
Acetone	0.50	0.650
Dioxane	0.50	0.409
Sulfolane	0.10	0.286
Acetone	0.10	0.131
Dioxane	0.10	0.083

As can be seen, the color intensity caused by sulfolane is far higher than that due to the other solvents tested.



Figure 4 shows the spectral absorption curves for the complex with sulfolane and with acetone. As can be seen, both maxima are at approximately 620 to 625 nm. Although the results of Table 4 compare reasonably well with those of Table 3, relative variations exist in the absorbances obtained with acetone, dioxane, and tert-butyl alcohol. These variations can probably be attributed to differences in solvent purity. West and DeVries did not state the purity of the solvents employed in their experiments. In the experiments conducted in this laboratory, analyzed reagent grade acetone, tert-butyl alcohol, and dioxane and J. T. Baker Chemical distilled sulfolane were employed. Lower absorbance values have been obtained with practical grade acetone. From the data it is apparent that the molar absorptivity of the complex under the given conditions with sulfolane is approximately 1400 liters per mole-cm. Further experimentation showed that in pure sulfolane solution, the molar absorptivity exceeds 2000 liters per mole-cm.

Attempts made to develop a complexometric photometric titration for cobalt utilizing the blue cobalt thiocyanate complex were only partially successful. Experiments employing solutions of tetraethylenepentamine (tetren) in sulfolane to titrate the Vogel Blue formed in sulfolane solution were performed. Difficulties in appropriately buffering the system and apparent decomposition of the cobalt tetren complex with time, caused this approach to be abandoned. It was found, however, that it is possible to titrate the thiocyanate complex with EDTA in 20 to 30 percent sulfolane in water solutions. In such a solvent mixture the molar absorptivity of the colored complex is reduced. In addition, since the molar absorptivity

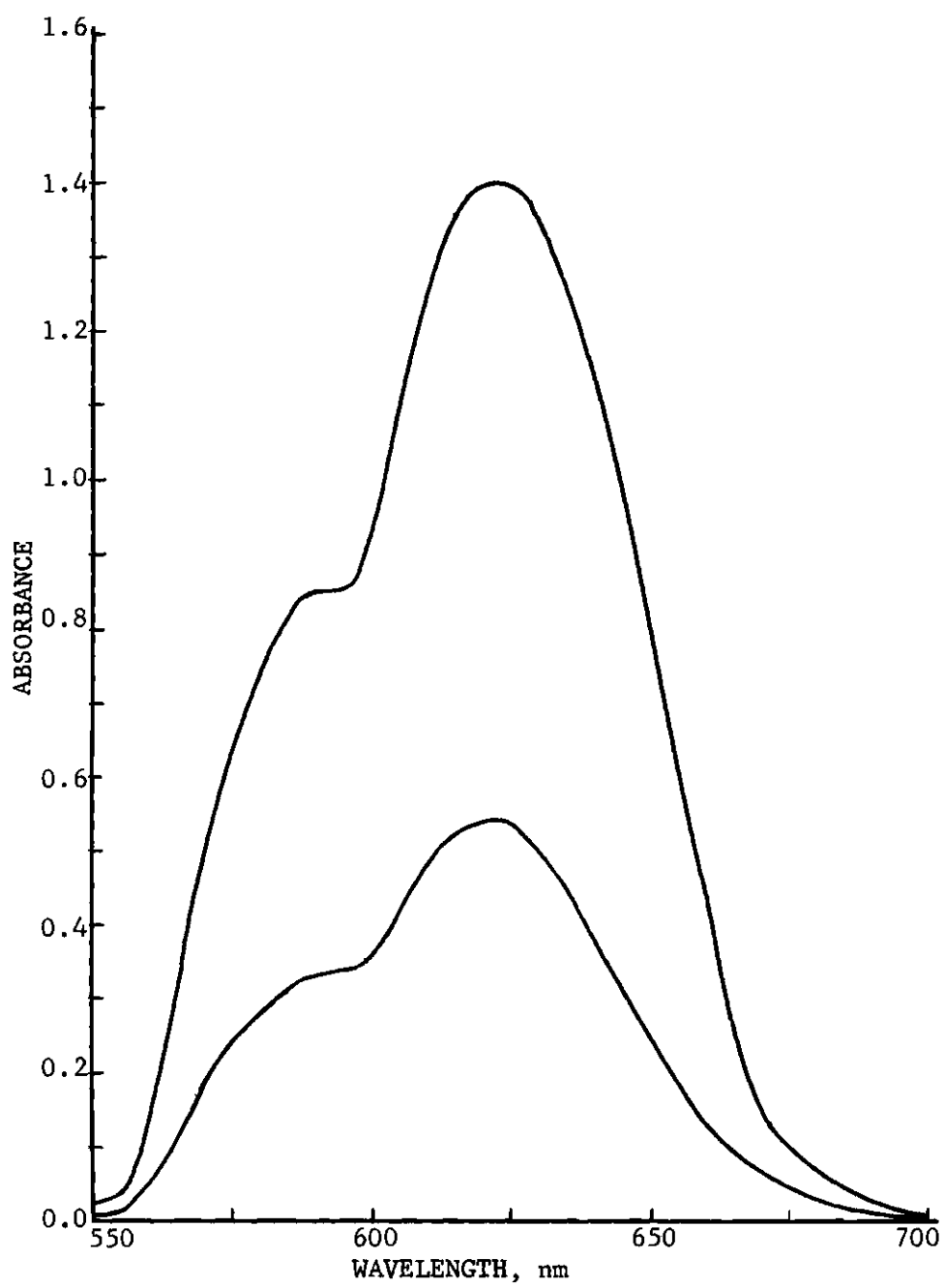


Figure 4. Spectral Absorption Curves of the Cobalt Thiocyanate Complex

of the complex increases rapidly with increasing sulfolane concentration (from about 200 liters per mole-cm in 20 percent sulfolane by volume to approximately 900 liters per mole-cm in 30 percent sulfolane in water solution) careful attention is required to insure that the concentrations of sulfolane in the titrant solution and solution to be titrated are identical. The thiocyanate concentration should also remain as constant as possible during the titration. These problems associated with the photometric titration indicated that a photometric determination is preferable to a phototitration.

#### Extraction of the Cobalt Thiocyanate Complex

As previously mentioned Young and Hall extracted the cobalt thiocyanate complex into a 3:1 mixture of amyl alcohol and ethyl ether. The extraction is performed at a pH of 3.5 to 4.0 with a high concentration of ammonium thiocyanate (26 percent solution). Specker and coworkers<sup>28</sup> extracted the complex into a mixture of ether and tetrahydrofuran from 3 molar hydrochloric acid solution. Under these conditions nickel is reported to be not extracted.

In both methods the acid serves to help enhance the formation of the blue complex and render it extractable (presumably by dehydration). Investigations of the complex in 40 volume percent sulfolane in water solutions, being  $2 \times 10^{-3}$  F in cobalt and 0.5 F in potassium thiocyanate indicated that the blue color is stable over a wide range of acidity, namely from highly acidic conditions to a pH of 9.0 to 9.5 where the hydrous oxide precipitates. Sulfolane thus appears to accomplish dehydration as does the acid.

It was felt that the complex developed with sulfolane might be employed in an extraction step to separate cobalt from nickel. Although nickel salts do form a green complex with thiocyanate the color intensity is low and the above indications of possibilities of separation encouraged the pursuit of the extraction.

After extensive experimentation with various solvents it was found that nitrobenzene was an excellent extracting solvent for the complex. In this work a Jarrell Ash Atomsorb Atomic Absorption Spectrophotometer was employed to determine the cobalt present in the extract and thereby test for the completeness of extraction. From solutions approximately 1  $\mu$  in potassium thiocyanate and containing 40 percent by volume sulfolane, cobalt (II) can be separated from a 500 fold amount of nickel by formation of the blue thiocyanate complex and extraction into nitrobenzene. This complex can then be subjected to photometric determination. Attempts to obtain effective separation with nickel to cobalt ratios higher than 500:1 failed because of incomplete cobalt extraction. Further work was abandoned when it became apparent that ratios comparable to those manageable with other methods developed in this laboratory<sup>29,30</sup> could not be attained.

#### Color Enhancement for Other Metal Complexes by Sulfolane

##### Thiocyanate Complexes of Other Metals

The possibility of enhancement of the thiocyanate complexes of other metals has been explored by Tice.<sup>31</sup> Sulfolane has been shown to enhance to colors of the thiocyanate complexes of Bi (III), Mo (V), and

W (VI). The enhancement in these cases, however, has been slight, with only about 1.5 times the normal absorbance being obtained with reasonable concentrations of sulfolane. It is worthy to point out that the color of the Fe (III) thiocyanate complex is not enhanced.

#### Color Enhancement for Chelate Complexes

Cheng and Goydich<sup>32</sup> reported that the color of gallium (III)-PAN species in aqueous solution shows a characteristic enhancement upon the addition of isopropyl ether in small amounts. It was logical to test sulfolane for enhancement in this system. However, no enhancement was found. The PAN complexes of Fe (III), Co (II), Ni (II), Zn (II), and Cu (II) were also tested. No significant color enhancement resulted from sulfolane addition. Similar tests conducted with PAR [4-(2-pyridylzo)resorcinol], SNAZOXs (8-hydroxy-7-[(4-sulfo-1-naphtyl)azo]-5-quinoline-sulfonic acid), xylenol orange, and calmagite also failed to show enhancement.

#### Conclusions

While the enhancement of thiocyanate complexes is certainly of analytical interest and sulfolane can be highly recommended as an excellent enhancing agent for the Vogel Blue complex, the levels of enhancement exhibited do not constitute the sensitivity increases desired. Enhancement of complexes of highest molar absorptivity would lead to significant improvement by providing sensitivity beyond current methods. Unfortunately no enhancement was observed with such complexes. Increasing the molar absorptivity of the thiocyanate complexes merely makes them somewhat more

competitive with sensitive color reagents but not to an extent of general practical significance. If, however, this increase in the molar absorptivity of the thiocyanate complexes due to sulfolane is employed in conjunction with the long-path microcells discussed in Chapters V, VI, VII, the increase in overall sensitivity may be a quite significant one. With this combination, the relatively selective Vogel reaction may be extended to use in a concentration range where it originally lacked adequate sensitivity.

## CHAPTER V

### THE DESIGN AND CONSTRUCTION OF A SPECTROPHOTOMETER INCORPORATING LONG PATH MICROCELLS

#### Optical Lay-out

Figure 5 is a general schematic representation of the optical lay-out of the instrument. Light radiating from the tungsten lamp and passing through the field lens and entrance slit is focused on the objective lens. The resulting collimated beam is directed onto the cam driven diffraction grating. Diffracted light reflected by the grating strikes the monochromator exit slit. The emerging monochromatic light beam is concentrated by a condensing lens onto the cell window and then passes through the cell to reach the detector.

A control comb and a shutter serve as regulating devices within the light path. The comb, positioned between the objective lens and grating, regulates the intensity of light striking the grating and can be used to adjust 100 percent transmittance. The shutter, located between the monochromator exit slit and the condensing lens, allows the light beam to be completely blocked from the detector and is used to set zero percent transmittance.

#### Photometer Construction

##### Base and Enclosure

All components of the instrument are mounted on a one inch thick

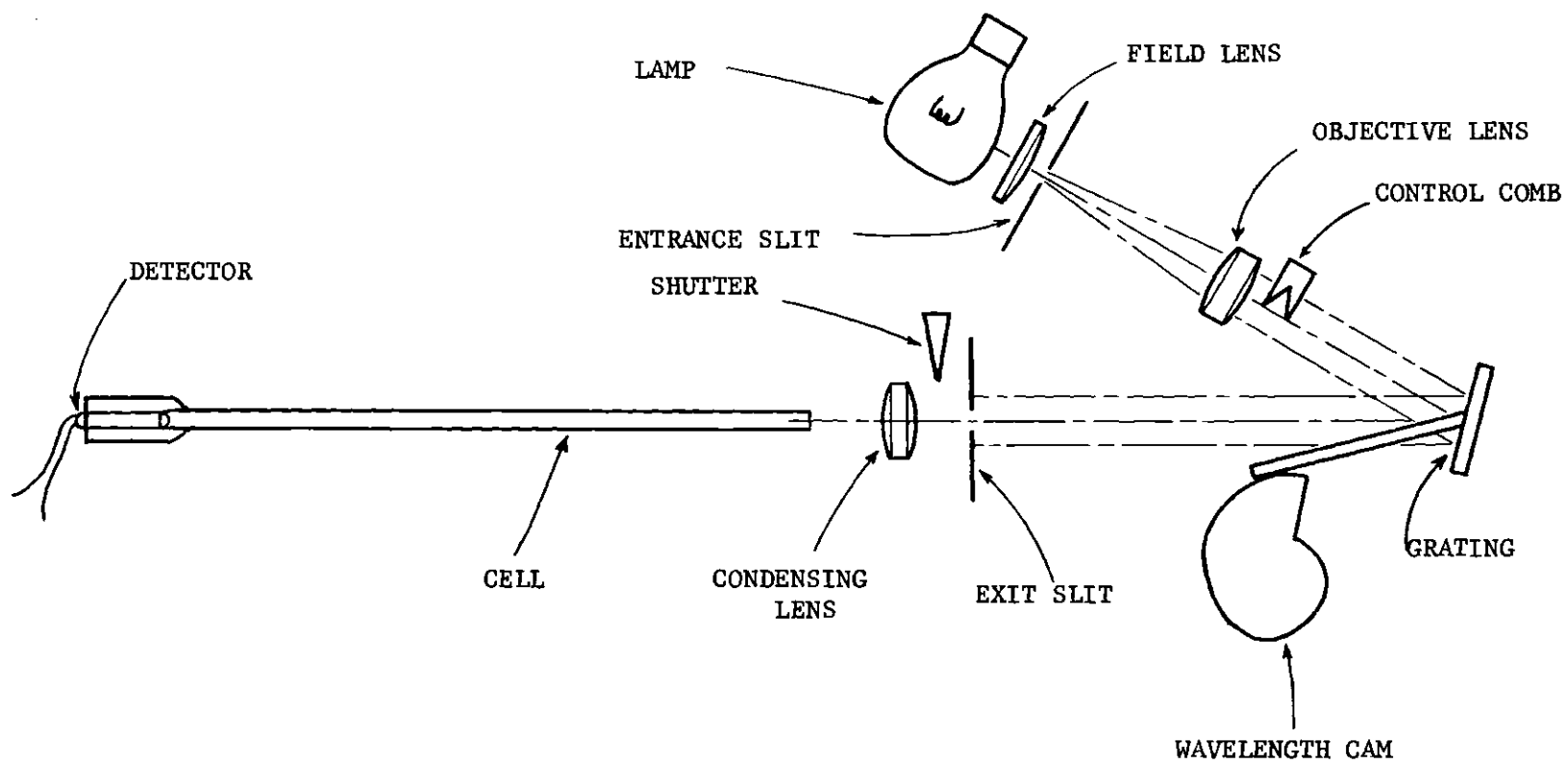


Figure 5. Optical System Schematic



plywood base which is cut in the form of an elongated hexagon of length 98 cm, width 32 cm, and two longest sides 58 cm. Aluminum plates are attached to this base where especially precise mounting is required. Heavy felt weather stripping is cemented around the edge to the underside of the base. This prevents rocking of the instrument, damps vibrations, and permits locating wires for electrical connections beneath the base.

Figure 6 shows details of the "light tight" instrument enclosure. The sides (12 cm in height) and top of the instrument are cut from black Plexiglas and screwed to the plywood base. They are readily removable to facilitate repair and adjustment. Black plastic tape applied to the outside of the corners insures complete exclusion of ambient light at the Plexiglas joints. The top overlaps the sides and is secured to six nylon posts driven into holes in the plywood base. Sponge rubber tape is applied around the inside of the walls at their upper edges. The tape projects slightly above the rim and provides a tight, even seal between top and sides. The top is equipped with a door 52 cm long and 10 cm wide to allow direct access to the cell. The door panel rests on the projected portion of felt tape, cemented to the underside of the top around the door opening.

Little attempt has been made to optimize to size or configuration of the enclosure. The elongated hexagon shape somewhat conforms to the optical arrangement. However, much internal space has been purposely left for future additions or modifications. In addition, electrical circuitry for the lamp power supply and photodetector circuit has been omitted from this enclosure to facilitate electronic and optical modification.

The components within the enclosure are arranged as shown in Fig-

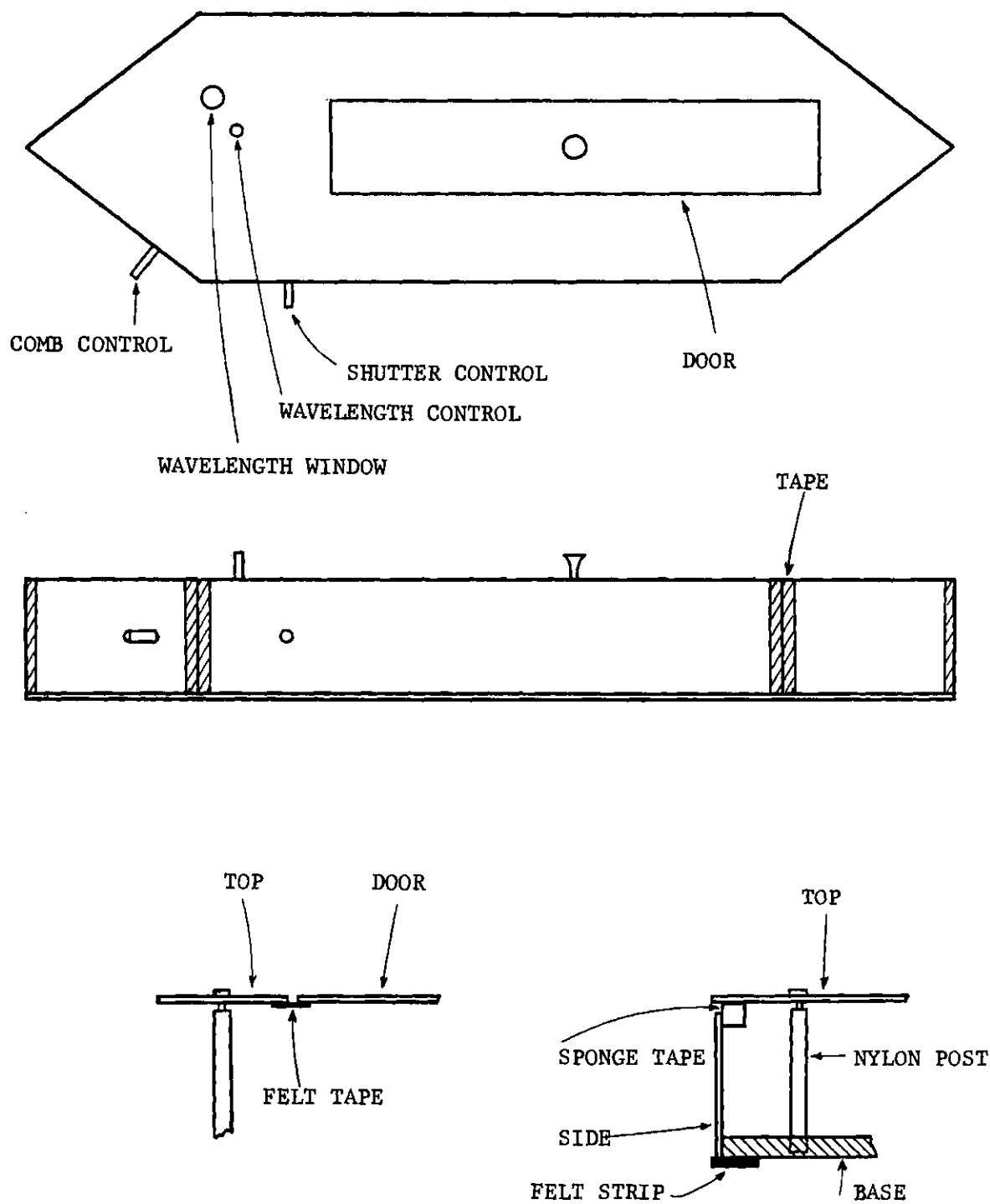


Figure 6. Instrument Enclosure

ure 7 and their constructional details are described below.

### Optical System

Construction of the optical system was facilitated by employing the diffraction grating with mount and integral cam follower arm, the wavelength cam, the collimating lens assembly, and several minor components from a Bausch and Lomb Spectronic 20. These components were selected because of their proven reliability, simplicity, and low cost. In the Spectronic 20, these components are mounted within fittings integral to a cast alloy base. Considerable construction was required to provide appropriate mounting for the components. Further, the instrument lay-out was modified to include a wide range of adjustment enabling precise alignment and thus maximizing the light passing through the small cell.

Light Source and Collimating Assembly. The light source, a General Electric 1631X instrument lamp, is mounted in a Bausch and Lomb Spectronic 20 spring loaded fixture which enables simple and accurate lamp replacement. The fixture is attached to a rack and pinion unit, in turn, rigidly mounted on a slotted adjustable bracket. The assembly is correctly positioned vertically and provides lamp adjustment within a plane parallel to the instrument base. Two set screws allow locking of the rack and pinion. The bracket is locked in place by firmly tightening the screws holding it to the instrument base. The light source and adjustable mount are housed in an aluminum box. The housing is a standard Bud (#CU-3008) Minibox cut to the desired configuration and fitted with felt tape around the edges. One end of the collimating lens assembly fits snugly into the housing and provides the only exit for the light. No ventilation openings

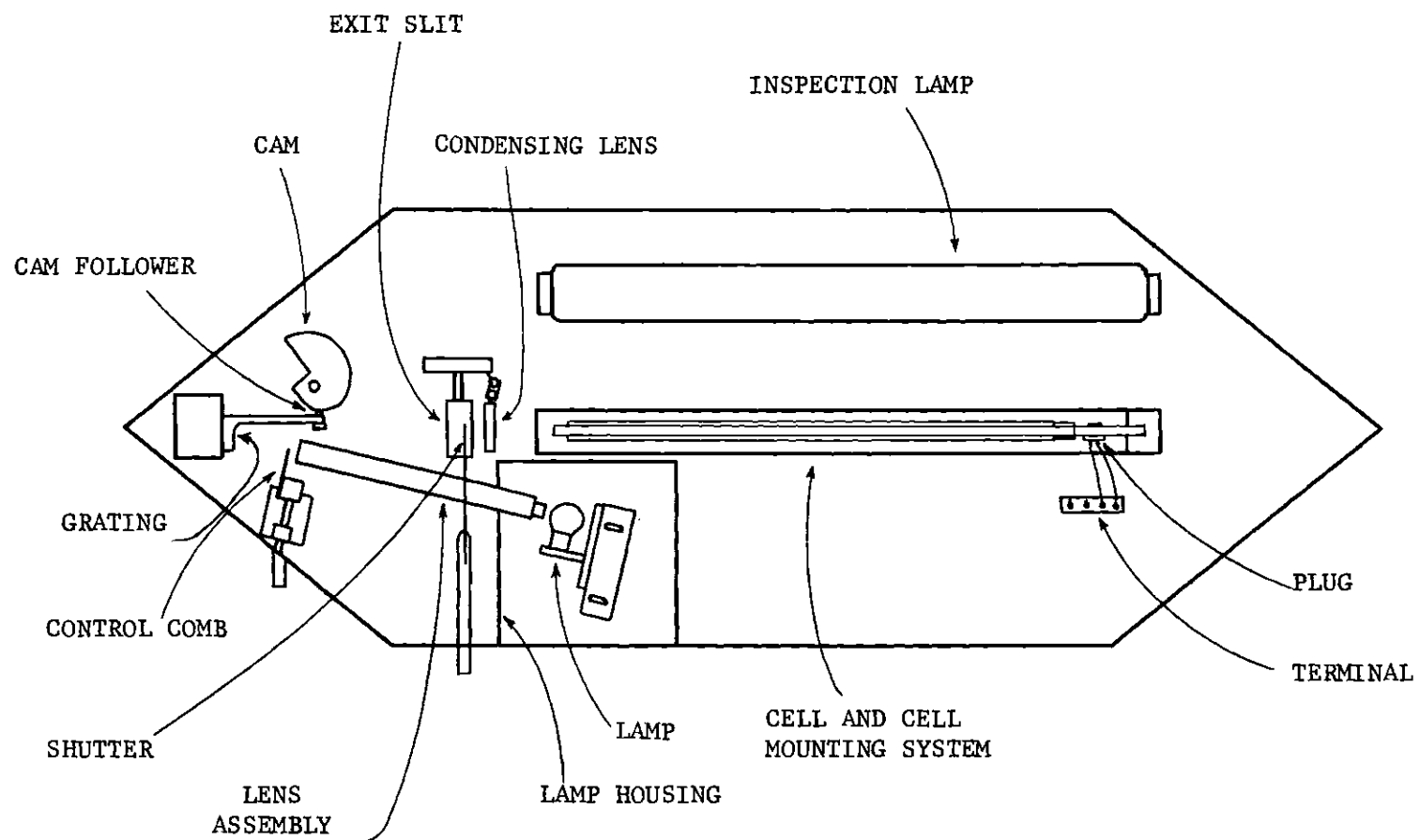


Figure 7. General Layout of the Instrument

are necessary since the heat generated is not excessive. The collimating assembly tube, itself, is securely mounted on an aluminum plate attached to the base.

Control Comb. The control comb is driven by a worm gear and adjustment is made by a knob located outside the instrument. The comb and gear were taken from a Bausch and Lomb Spectronic 20 no longer in service, but have been modified and remounted for this application.

Grating and Cam Assembly. The diffraction grating, with mount and cam follower is secured in a cage type housing where it rotates between two steel shot. The bottom plate of the housing is slotted and secured to the base with four screws. The slots allow slight shifting of the housing to achieve correct alignment. The cam follower is threaded into the end of the cam follower arm and locked with a lock nut. This threaded shank provides the adjustment required when calibrating the monochromator. The shaft of the cam driving the grating is seated in a mounting bracket with bronze bushing machined especially for the cam. A pointer to indicate the wavelength setting of the monochromator is also attached to this mounting bracket. A Bausch and Lomb Spectronic 20 wavelength scale is fixed on the threaded cam shaft with a nut and lock washer. The wavelength setting is read through a window in the instrument top. The cam shaft projects through the top where a control knob completes the cam assembly.

Monochromator Slits and Shutter. The monochromator entrance slit is actually located within the tube of the collimating lens assembly (see Figure 5). The exit slit was constructed from two razor blade edges held in position by a brass frame. The slit width is 1.5 mm at present, but

can be adjusted by shifting one of the razor blades. The frame is positioned within a track of two rails in which it is horizontally adjustable for proper optical alignment with the cell by a screw drive mechanism. The shutter was cut from brass sheet stock and slides within a dovetail in the frame of the exit slit. Shutter control is effected via a shaft extending through the wall of the instrument with an attached push-pull knob. Connected to the exit slit assembly between the slit and the cell, is a condensing lens that concentrates the light on the cell window. This achromatic lens is cemented to a circular Plexiglas frame attached to a locking double ball joint clamp. The ball joints enable accurate positioning of the lens to maximize the light reaching the cell window.

#### Cell and Cell Mounting System

Photometric Cell. Figure 8 shows details of the cell and cell mounting system. The cell is constructed of Pyrex brand glass tubing. Two sizes of tubing have been employed for cell body construction: 3 mm O.D., 0.6 mm wall and 2 mm O.D., 0.5 mm wall. The body tubes are 40.0 cm in length and are fitted with 2 mm O.D. inlet and outlet tubes 1 cm long, at the ends. Each body tube is joined to a short section of 4 mm O.D., 0.8 mm wall Pyrex tubing. This 1.5 cm long, flared portion of each cell accommodates the photoreceptor, a Texas Instruments IN2175 photo-duo-diode. The photoreceptor is wrapped in Teflon tape and permanently cemented into the flared tube end with epoxy adhesive. The opposite end of the cell is fitted with a small window, fabricated from a microscope cover glass. The window is cut to the appropriate size (about 3 mm by 6 mm) and, except for a circular center portion, is etched with hydrogen fluoride to improve adhesion. The window is glued with epoxy to the end

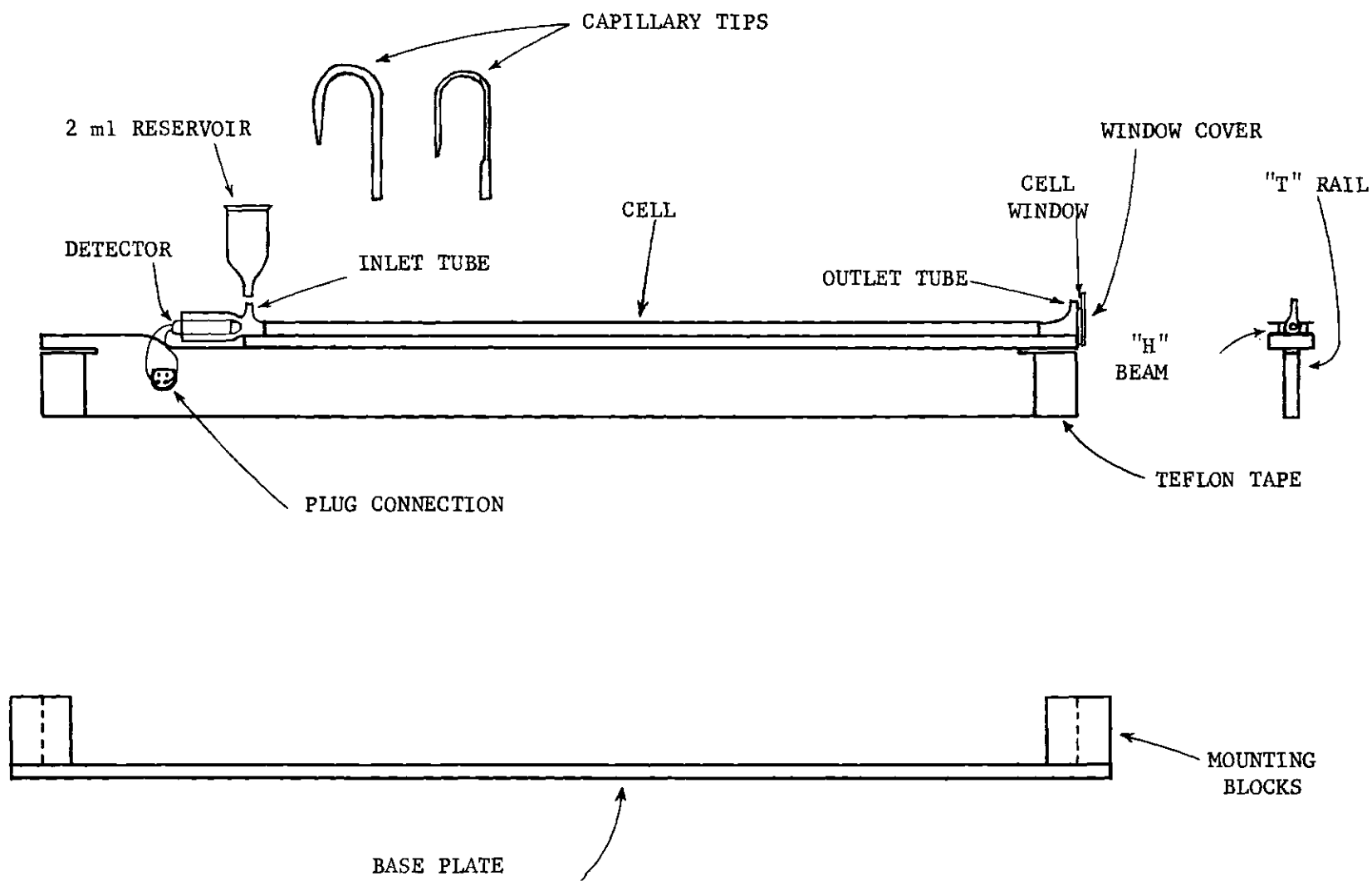


Figure 8. Cell and Cell Mounting System

of the cell and the cell mount, flush with the tube end. A diaphragm consisting of a gray plastic wafer (4 mm by 12 mm) with a hole of the same diameter as the inside of the cell tube is cemented over the window so that hole and tube bore coincide. The diaphragm prevents light from frontally entering the walls of the tube.

The cell is positioned between two "H" shaped plastic beams as used in model construction. The beams are 37.5 cm in length and cemented to the flat top of the "T" rail (see below) thereby clamping the cell firmly to the mount. Effecting this connection is greatly facilitated by the use of Tescom Zipbond, an ultra-fast drying contact adhesive.

Cell Mount. The cell mount, constructed from 1/8" clear and colorless Plexiglas supports the cell. The mount is made by cementing a Plexiglas strip 38 cm long and 0.8 cm wide to the top edge of a second strip 48 cm long and 2.5 cm high to form the "T" rail. The mount is securely positioned with proper alignment in the following manner. Two mounting blocks are provided each having a slot to receive the ends of the cell mount. For a snug fit in the slots, Teflon tape is wrapped around the ends of the "T" rail as shown in the diagram. The slotted blocks are fabricated from Micarta and glued to a plastic base plate providing a smooth mounting surface. The "T" rail and mounting unit are aligned with the monochromatic light beam by maximizing photodetector response. The plastic base plate with mounting blocks is then secured to the instrument base with screws.

The leads from the photodetector are soldered to a specially constructed plug connection, secured in a hole through the cell mount. A mating plug is attached with short wires to a terminal in the instrument base.



### Inspection Lamp

To enable the filled cell to be checked for air bubbles and particulate matter, an inspection lamp is provided. This is a 15-watt fluorescent tube located alongside of the cell mount (Figure 7). To provide appropriate cell illumination, the bottom of the "T" rail and the side farthest from the inspection lamp are painted with white enamel. With this arrangement light is guided up through the cell allowing simple inspection of its contents.

### Electrical Circuitry

Wires for electrical connections to the photoreceptor base terminal, the light source, and the fluorescent inspection light are made beneath the instrument base. Controls and circuitry for these components are contained in separate metal boxes to reduce mutual electrical interference and enable simpler modifications or repair of the various units.

### Photodiode Circuit

The circuitry employed for control and amplification of the photoreceptor output is shown in Figure 9. The major components of this circuit are three Fairchild integrated circuit operational amplifiers A1, A2, and A3 (one  $\mu$ A725 and two  $\mu$ A741, respectively) and one Analog Devices 751P logarithmic module. The module includes two silicon transistors ( $Q_1$  and  $Q_2$ ) and a temperature compensating voltage divider (resistors  $R_{TC} = 995 \Omega$  and  $R_G = 15 K\Omega$ ). Three 7 volt mercury batteries provide the power for the photodiode and two identical batteries serve as power supply for the operational amplifiers. The control elements are an on-off switch, S1; a four position mode switch, S2; two potentiometers, P1 and P2, to set 0 and

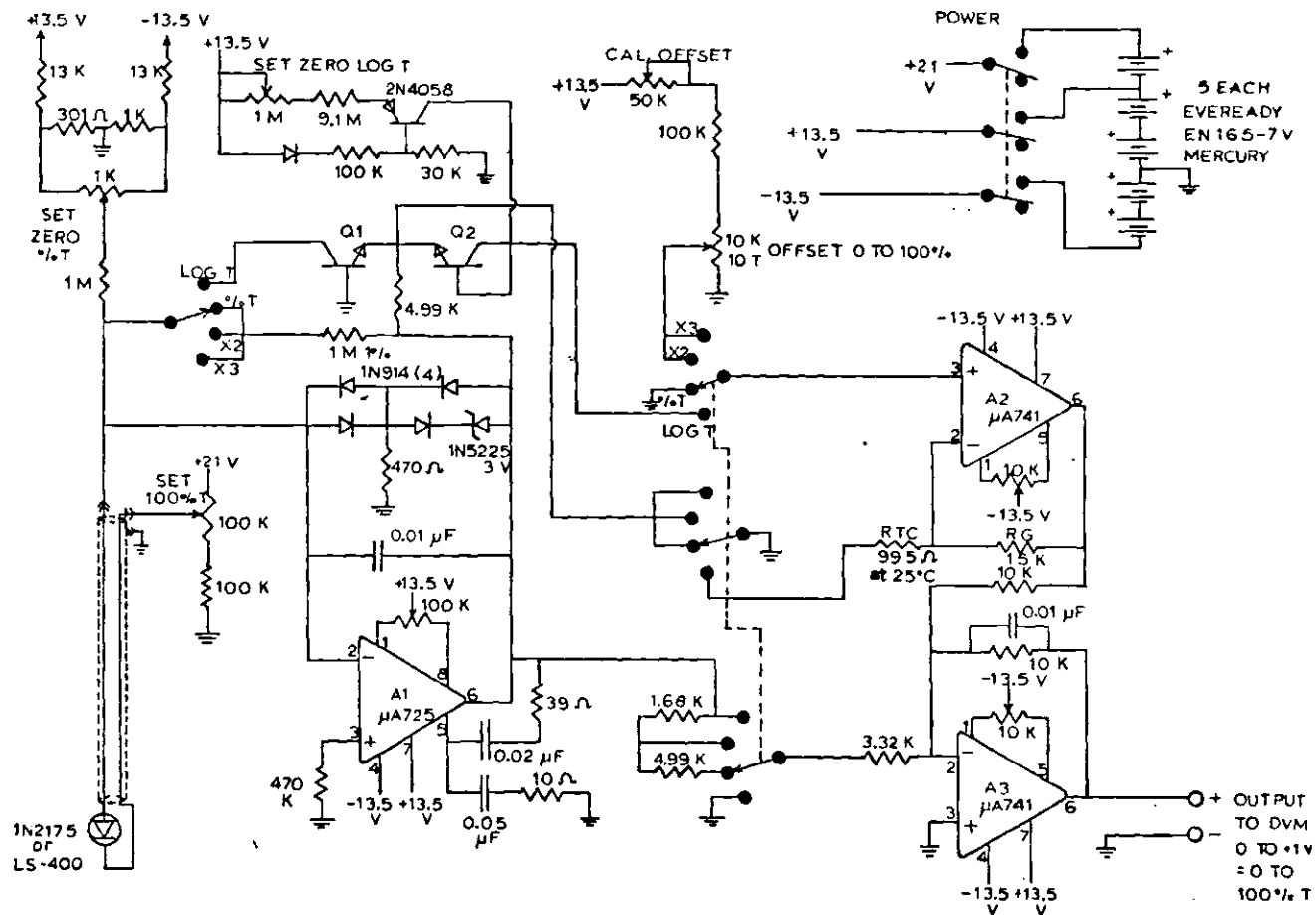


Figure 9. Photodiode Circuit

100 percent transmittance, respectively; and a potentiometer, P3, for voltage offset. The mode switch has positions of "% T", "% T  $\times$  2", "% T  $\times$  3", and "log T." The voltage offset control is equipped with a ten-turn counter dial. Potentiometers P4 through P8 serve to internally calibrate the circuit functions. A procedure for the internal calibration is provided in Appendix 2. All components except the control elements are soldered to an etched circuit board. The entire circuit is enclosed in a Bud (#CU-3008) Minibox and connections to the photodiode are made with a locking five-prong plug and socket. Connection is made by a shielded cable with a free-floating ground on the photodiode end. The 0 to 1 volt output of this unit is displayed on a Keithley Model 160 Digital Multimeter.

A circuit as just described, involving minute currents and logarithmic operation, is subject to extreme temperature sensitivity and dynamic instability unless certain design precautions are taken. The design thus required very specialized knowledge and was left to Mr. G. O'Brien, the electronics expert of the Georgia Tech Chemistry Instrument Shop.

#### Power Supply for Photometer Lamp

Power for the light source may be provided by two or more 6 volt automotive storage batteries connected in parallel. However, with the 1631X lamp employed in this instrument, frequent recharging of the batteries is required. Therefore, the use of an electronic power supply seemed more advantageous. A schematic of the circuitry for a highly stable power supply, especially designed for the application at hand, is given in Figure 10. Like the photodiode circuit, the layout of this unit is subject



to high temperature sensitivity. Consequently, this special circuit was also designed by Mr. G. O'Brien. The lamp switch, LS, activates the supply and the voltage applied to the lamp. At the power supply terminals 5 to 7 volts D.C. and 0 to 3.5 amps are available. The normal load with the 1631X lamp is 6.5 volts and 2.75 amps. The lamp supply is equipped with an input for automatic intensity regulation, AR. This input can be used in conjunction with another photodiode to provide additional stabilization (essentially a semi-double beam configuration). The entire circuit is enclosed in a Bud (#WA-1540) cabinet.

#### Inspection Lamp Circuit

The fluorescent inspection lamp is operated by a standard button switch and transformer taken from a discarded desk lamp. These components are encased in a Bud (#CU-3004-A) Minibox and connected to the instrument by a plug and socket junction.

## CHAPTER VI

### DISCUSSION OF THE PHOTOMETER DESIGN

#### General Design Approach

The simple, single-path design just described was pursued only after considerable experimentation and deliberation with other possible approaches.

Initial experiments were concerned with the Duboscq colorimeter principle. It was felt that small fiber optic-bundles within the cell tubes might be employed in adjusting the path length to obtain a balance in intensity of light beams emerging from two separate tubes containing standard and sample solutions. While it is easy to envision a precise electronic null system employing two photodiodes, and accurate measurement of sample concentration from the standard concentration and two path lengths, results of preliminary investigations caused this approach to be abandoned. With fiber optics, significant light is always lost at the ends of the bundle due to improper focusing of the incident beam on the individual fibers and high dispersion of the emerging beam. Without special focusing of the light emerging from the fiber bundle, the obtained absorbance readings were found to be nonlinear with respect to path length. In addition, flexing of the fiber bundle caused difficulties by altering the direction of the emerging light beam and also complicated measurement of the path length.

With these considerations it seemed to be more accurate to measure

the absorbance directly, electronically, instead of employing null detection and measuring path length. However, the decision whether to make the instrument single beam or double beam was still required. The primary advantage of the double beam principle is compensation for instability of the light source and certain other components. With the provision of a sufficiently stable lamp supply, the simpler single beam design was more appealing.

#### Photodetector Considerations

It is known that phototransistors generally provide greater sensitivity than photodiodes. Initially, with the proposed design utilizing fiber optics, this sensitivity was required. However, without the light losses associated with fiber optics, the available phototransistors lost much of their desirability for the intended application. Certain small photodiodes offer good sensitivity while showing greatest adaptability to the present purpose. Such silicon photo-duo-diodes as the Texas Instruments IN2175 and LS-400 are equipped with an integral lens. The light sensitive semi-conductor chip is located near the focal point of that lens. Light not entering within an extremely small angle about the optical axis entirely misses the chip. This small angle of acceptance is especially desirable in eliminating extraneous light interference. The shape and small size of these devices (12 mm in length by 2 mm in diameter for the IN2175 and 16 mm in length by 2 mm in diameter for the LS-400) are of value in their alignment with the bore of photometric cell. Both devices have been shown to exhibit the qualities desired in instruments previously constructed in this laboratory.<sup>33,34,35</sup> With 21 volts provided

by three mercury batteries of 7 volts each, the LS-400 shows about 5 times greater sensitivity than the IN2175 and is therefore employed with the smaller (2 mm O.D., 0.5 mm wall) cell. Both photodiodes have adequate sensitivity throughout most of the visible range. The sensitivity, however, decreases rapidly with shorter wavelengths and is very low below 420 nm. With the reduced energy output of the light source at lower wavelengths, work in the near-ultraviolet region is impossible. This problem can, perhaps, be remedied by the use of a lamp emitting more light in the shorter wavelength range.<sup>36</sup>

Three controls are included to compensate for the varied spectral response of the photodiode:

1. A variable load resistor at the output of the lamp supply
2. A comb to adjust light intensity
3. Resistors in the mode switch to provide three amplification factors.

The first two controls enable exploitation of almost all of the visible spectrum. For work in the blue-violet and violet regions, additional amplification is available. In addition, with the Keithley Digital Multimeter, a higher numerical read-out may be obtained by simply switching from the 0 to 1 volt range to the 0 to 100 millivolt range.

#### Signal Readout

While the Keithley Digital Multimeter provides rapid reading and is invaluable in instrument development, it is not required for operation of the instrument. The photodiode circuit has been designed to be employed with any standard voltmeter capable of accurate measurements in



the 0 to 1 volt range. Since low-cost voltmeters with 0 to 1 volt full scale deflection are uncommon, it may be preferable to use a current meter. A good quality meter with a full scale current of from 10 microamperes to 10 milliamperes would be satisfactory. In utilizing a current meter with the instrument, it is necessary to use a resistor,  $R$ , in series with the meter. The value of the resistor in ohms may be computed from

$$R = \frac{1 \text{ volt}}{I_m} - R_m$$

where  $I_m$  and  $R_m$  are the full scale meter current in amperes and the internal meter resistance in ohms, respectively.

#### Photodiode Circuit

Initial experiments were conducted with a circuit similar to those used in previous photometers and phototitrators designed in this laboratory.<sup>33,34,35</sup> This simple circuit is shown in Figure 11. The electric circuit illustrated was specifically designed for use with a Honeywell Rubicon galvanometer with a sensitivity of about 0.0005  $\mu\text{A/mm}$  over the 100 mm scale, a critical damping resistance of 57 kilohms, and an internal resistance of about 4.4 kilohms. Basically, the complete circuit is a series arrangement of the photodetector, photodetector power supply, a zero suppression circuit, and a zero adjust network. Increased sensitivity may be obtained by proper programming of the zero suppression circuit. The zero adjust provides dark current compensation and enables a simple adjustment of the zero point of the galvanometer.

While the circuit performs its design functions well, it is

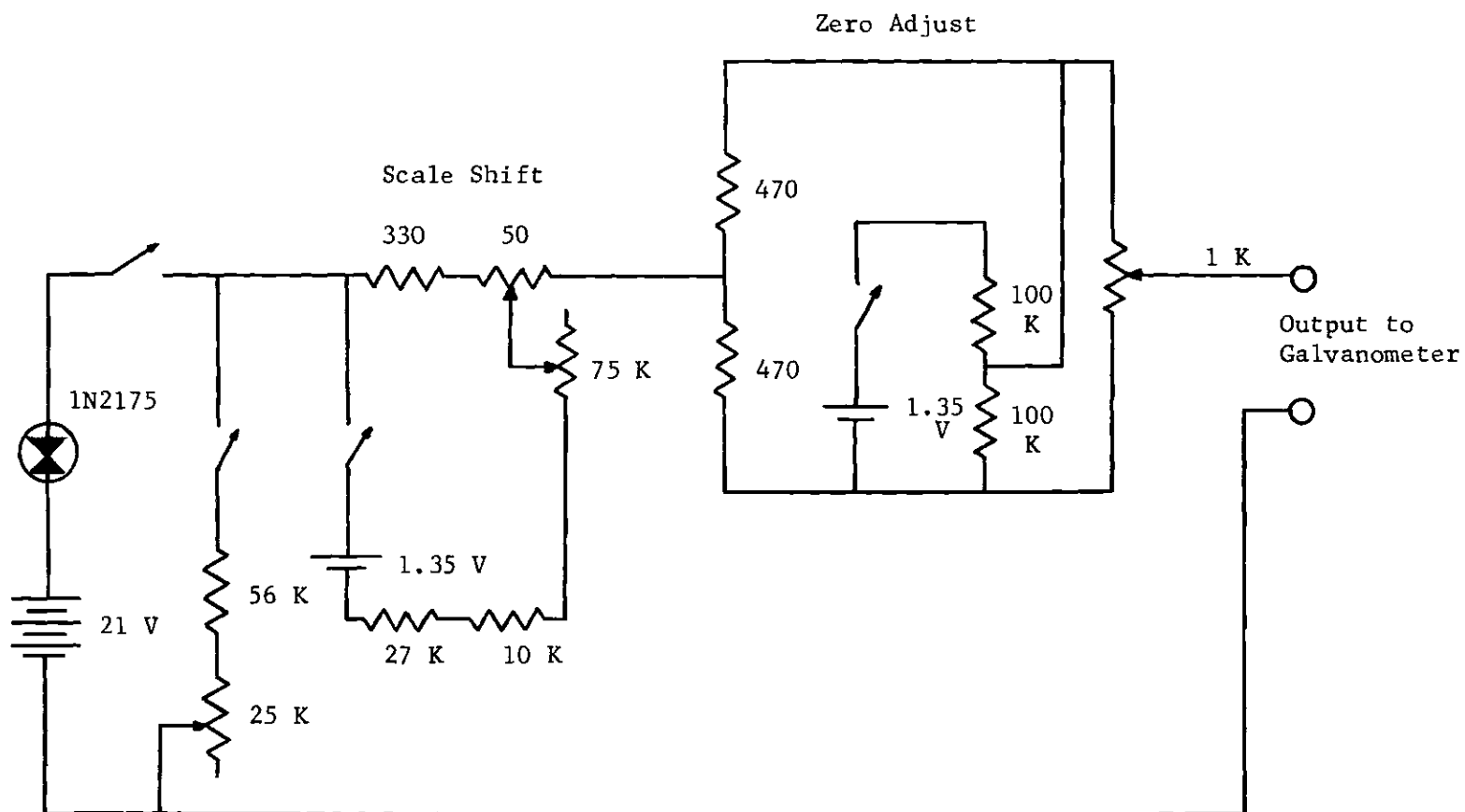


Figure 11. Initial Photodiode Circuit

strictly limited to use with the very sensitive Honeywell Rubicon galvanometer described. No provision for a logarithmic output for absorbance readings has been made and no amplification of the photodiode signal is available. Adjustment of the zero percent transmittance setting is precise and accurate. However, setting of 100 percent transmittance with a light intensity comb and variable resistance in series with a lamp supply battery is often aggravating because of the lack of precision and the nonproportionality in these controls. Further, the 100 percent transmittance may drift until a thermal equilibrium has been reached by the voltage-regulating resistance. The present photodiode regulation circuit provides precise setting of both zero and 100 percent transmittance. The control comb and lamp voltage regulation are normally applied for an only approximate setting of 100 percent transmittance.

#### Cell Alignment

The instrument has been designed to accommodate various sample cells without optical realignment. The key to realization of this feature is the use of a unit cell and cell mount, fitting into slotted blocks. In addition, even after condensing the monochromatic light beam with the lens, the light beam is of sufficient size so that slight variation in cell position can have no ill effects. The incorporation of an individual photodiode, permanently fixed to each cell, is another important factor in maintaining proper alignment.

## CHAPTER VII

### OPERATION AND EVALUATION OF THE PHOTOMETER

#### Instrument Operation

At this point it is desirable to note that the instrument may be employed as a phototitrator in addition to standard photometer operation. Only simple modifications of the top door, to accommodate the buret tip and suction tube, and addition of a syringe are needed. With regard to instrument operation, essentially the only difference is that precise setting of 100 percent transmittance is usually not required for photometric titrations, while operation as a photometer necessitates exact setting of 100 percent transmittance. A general description applicable to either method of operation is provided below.

#### General Operating Procedure

Filling the Cell. For most applications a 2 ml capacity reservoir such as illustrated in Figure 8 is attached to the inlet tube of the cell with a short section of rubber surgical tubing. The cell outlet tube is attached via surgical tubing to a simple liquid trap made from an Erlenmeyer flask. The outlet of the trap is equipped with a third section of rubber tubing to which is added a small glass mouthpiece or the hypodermic syringe. Filling of the cell is then accomplished by applying a slight suction with the mouth or syringe.

It is recommended that the cell be flushed, first with several

portions of reagent grade acetone or ethanol and then with distilled water or appropriate solvent blank to clean it prior to introduction of the blank for setting of the instrument. Upon introduction of the blank, standard, or sample solutions the inspection lamp is switched on and the cell contents are examined for bubbles and particulate matter. The inspection light is, of course, switched off before measurements are made.

Adjustment of 0 and 100 Percent Transmittance. Approximately ten minutes is required for proper stabilization of the light source power supply. Consequently, the light switch is usually activated before cleaning and filling the cell.

With the lamp supply sufficiently stabilized, the cell filled with blank solution and inspected, and the top door closed, the instrument is ready for setting 0 and 100 percent transmittance. This operation proceeds as follows:

1. Select the desired wavelength for the photometric measurement with the wavelength control.
2. Set the range switch of the digital voltmeter on the 0 to 1 volt range and activate the meter.
3. Switch the photodiode circuit to the "ON" position and set the mode selector switch to "% T."
4. Close the optical shutter and adjust 0 percent transmittance (0.000 volts) with the zero-set control.
5. Open the shutter and roughly set 100 percent transmittance (1.000 volt) using the lamp voltage control and the light intensity control comb.

6. Make the fine adjustment of 100 percent transmittance with the "100 % T set" of the photodiode circuit.

7. Close and reopen the shutter, checking the 0 and 100 percent transmittance settings, respectively.

When operating below about 460 nm, where the sensitivity of the IN 2175 (or LS-400) photodiode is low, the digital voltmeter may be set on the 0 to 100 millivolt range or the photodiode circuit set on the "% T X 2" or "% T X 3" position to enable meter readings of 100.0 millivolt or 1.000 volt equal to 100 percent transmittance, respectively. With the mode switch in the "% T X 2" or "% T X 3" positions, the "100% T set" control is ineffective and 100 percent transmittance must be adjusted by carefully turning the knob of the light intensity control comb.

Measurement of Standard and Sample Solutions. After appropriately setting 0 and 100 percent transmittance with the shutter and blank solution, the standards and sample are introduced as described for the blank solution. It is best to first wash the cell with two small portions of the solution to be measured before filling for taking the reading. The readings can be taken as percent transmittance or the mode switch can be alternatively switched from the "% T" position to the "log T" position and the logarithm of the transmittance (negative absorbance) obtained directly, over three decades of transmittance values.

#### Voltage Offset Control

The voltage offset control enables shifting of the scale zero in the negative direction, a desirable feature in applications where higher precision is required. This increased sensitivity by zero suppression is especially applicable when a standard, left-zero, deflection meter is

employed instead of a digital voltmeter. The control is effective only in the "% T X 2" and "% T X 3" positions. The scale length is increased by setting the number of additional scale units desired (from 0 to 100) on the voltage offset control counter-dial and adjusting the light intensity with the supply voltage control and intensity control comb to obtain a 100 percent scale reading. The effective zero is then displaced down scale (negative) the number of scale units shown on the ten-turn counter dial. For readings below the meter zero the voltage offset is returned to the zero position and the effective zero and meter zero again coincide. With a three and a half digit readout on the digital voltmeter, a larger scale range can be obtained by simply increasing the light intensity (on any of the percent transmittance mode positions) to give a meter reading up to the limit (1.999 volt).

Regardless of the mode of operation, the "0% T set" should be adjusted with the mode selector switch in the "% T" position to give a reading of 0.000 volt.

#### Phototitrator Operation

As previously mentioned, the operation as a phototitrator is essentially identical with photometer operations. While operation as a photometer requires exact settings of 100 percent transmittance, operation for photometric titration does not require an exact setting. The upper limit can, in fact, be any appropriate value within the range of the instrument and meter (with or without a scale shift).

One use for the instrument in photometric titrations is simply to monitor the change in absorbance of a solution being titrated externally

and separate from the instrument. For this type of operation one of the capillary tips (Figure 8) can be employed on the cell inlet tube to facilitate the introduction of portions of the solution during the titration. Use in this manner is applicable to larger volumes of solution with low absorbances. In addition, as a "true" phototitrator, the instrument can be connected with rubber or plastic tubing to the external titration vessel and a peristaltic or vibrastaltic pump can be employed for circulation of the solution through the cell.

The application most in keeping with the small volume capacity is to conduct the titration within the small reservoir attached to the inlet tube of the cell. For this method of phototitration, the top door modifications and syringe, as previously indicated, are needed. Homogeneity is obtained after each addition of titrant by repeatedly pulling the solution into the cell and pushing it back into the reservoir with several strokes of the syringe, set for this purpose to the required stroke length. If the volume of the solution to be titrated is very small, it is important to dry the cell by first flushing with acetone and then pulling air through it. The solution to be titrated is then added with its volume measured so that proper correction for the dilution due to titrant addition can be made. Such considerations need not be applied if a titrant of sufficiently high concentration, relative to that of the solution being titrated is used so that the volume change during the titration is less than one percent. In such cases, the titrant is added by means of a precision micro buret.

With regard to instrumental technique, two principal cases of



phototitrations may be differentiated; a down-scale titration, toward lower scale readings, and an up-scale titration, toward higher scale readings.

Down-Scale Titrations. Here the sample solution is introduced into the reservoir and cell, and the light intensity adjusted with the lamp voltage control or light intensity control comb to bring the meter reading to the 100 (or other appropriate) mark. When this is accomplished, the titration is started and readings taken after each addition of titrant. Increased sensitivity may be secured if desired by employing the voltage offset control for zero suppression.

Up-Scale Titration. This type of titration can present problems because it requires knowledge of the approximate meter reading at the end of the titration. If zero is adjusted as usual and the solvent is used to set 100 percent transmittance, the meter reading will definitely remain on scale during the titration but the high sensitivity capability of the instrument is not fully utilized. It is much better to adjust the 100 percent scale reading with an over-titrated solution to utilize the maximum change in readings during the titration. It is usually desirable to adjust the meter reading to 95 or even to 90 in order to allow for the increase in transmittance due to dilution during the titration. Alternatively, adjustment to a reading of 100 may be made on the "% T X 2" or "% T X 3" mode and the voltage offset used to shift the scale if meter deflection exceeds the full scale reading.

## Performance Tests

### Wavelength Calibration

Interference filters with sharp maxima were used to calibrate the wavelength indicator scale. An interference filter was placed in front of the cell window and the cam rotated to give maximum detector output. The peak transmission wavelength of the filter is then set on the indicator scale and the scale locked into place on the threaded cam shaft with the nut and lock washer. Fine adjustment of the setting was made using the threaded cam follower. The calibration conducted in this manner was verified with six additional interference filters with different peak transmission wavelengths between 420 and 700 nm. All of these filters read within 3 nm of their labeled values, indicating a high level of agreement between the wavelength scale reading and the wavelength of the light striking the detector.

### Stability and Independence from Stray Light

With sufficient warm-up of lamp and lamp power supply, the instrument showed only minor short term fluctuations ( $<0.001$  volt). No significant long term drift was observable. With the top door correctly closed bright external light has no effect on the instrument readings and less than 0.2 percent transmittance change was observed in the zero setting (with the shutter closed) when the lamp power supply was switched off and on. With the Keithley Digital Multimeter the instrument showed readings of reasonable stability (2 to 3 percent transmittance) on even the 0 to 10 millivolt scale.

When fluctuations do occur, they can usually be traced to particulate matter suspended in the optical path outside of or within the cell.

Occasionally, line voltage fluctuations cause some erratic readings, probably by affecting both the multimeter and lamp power supply. On a well regulated line, the lamp power supply shows stability within a millivolt and the multimeter is stable within  $\pm 0.1$  percent full scale when on the 0-1 volt, 0-100 millivolt, and 0-10 millivolt ranges.

#### Cell Alignment

Removal and replacement of the cell and cell mount unit generally results in changes of less than 0.2 percent of the 100 percent transmittance setting.

Although the detector output can be maximized for each separate cell by readjusting the condensing lens, this adjustment is not required to obtain an adequate detector response when the cell is exchanged. If the detector response of the cell with the large body tube (3 mm O.D., 0.6 mm wall) is maximized and the smaller cell (2 mm O. D., 0.5 mm wall) is substituted the coincidence of the light beam with the cell bore will remain satisfactory.

#### Linearity of Response

The linearity of the instrument's indicated response was tested with screens according to a method previously described.<sup>37</sup> A Cary 16 spectrophotometer was employed to first measure the absorbance of the screens and these absorbance values were plotted versus those obtained with the instrument. Two separate experiments were conducted involving two different locations of screen placement within the light path of the instrument. In the first case the screens were located directly against the cell window. This location led to variation in readings with the

position of the screen holes over the small aperture of the cell bore. Thus, several readings were taken, shifting the screen between readings. In the second case the screens were placed directly in front of the monochromator exit slit. Both experiments gave straight line plots through absorbance values of 1.6 (the maximum of the screen set used) with a slope less than one. The slope of less than unity indicates that the image of the screen is generally diverging between the screen and the detector.

These tests were taken as a strong indication that, as a unit, the photodiode and photodiode circuit respond linearly.

#### Testing of Operational Modes

The various operational modes of the photodiode circuit were tested for their internal consistency and for accuracy relative to each other.

"Log T" (negative absorbance). The logarithmic mode was tested for accuracy and calibration by adjusting various percent transmittance values in the "% T" mode and switching to the "log T" mode to obtain the corresponding logarithmic reading. After proper calibration (see Appendix 2) the following results were obtained:

$$1.000 \text{ volt} = 100.0 \% T$$

<u>% T</u>	<u>log T</u>
100.0	0.000 volts
10.0	-1.000
1.0	-1.999
0.1	-3.00 (DVM on 10 volt range)

"% T X 2" and "% T X 3". The "% T X 2" and "% T X 3" positions were checked for accuracy relative to the "% T" position of the mode switch. The following corresponding readings were obtained:

$$1.000 \text{ volt} = 100.0 \% T$$

<u>% T</u>	<u>% T X 2</u>	<u>% T X 3</u>
0.0	0.0	0.1
25.0	50.0	75.2
33.0	66.7	100.2
50.0	100.0	150.3

#### Voltage Offset Control

The accuracy of shifting the scale with the voltage offset control was tested by observing the number of turns of the counter dial required to bring about a given change in the percent transmittance reading. The position of the shifted-scale zero was also checked by closing the shutter and reading the negative percent transmittance in volts from the digital voltmeter. The following are typical results:

<u>Initial %T</u>	<u>Final %T</u>	<u>-Δ%T</u>	<u>Counter-Dial Reading</u>	<u>Zero* Reading</u>
100.0	90.0	10.0	10.0	- 10.0
100.0	80.0	20.0	20.2	- 20.1
100.0	50.0	50.0	49.7	- 50.1
100.0	20.0	80.0	80.1	- 80.3
100.0	00.0	100.0	99.5	-100.3
120.0	100.0	20.0	19.8	- 20.1
60.0	50.0	10.0	10.3	- 10.2

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\* Although the photodiode circuit has not been designed to give linear readings below zero percent transmittance, the linear range appears to extend to 100 percent transmittance in the negative direction.

#### Measurement of the Effective Path Length

Before proceeding with this discussion it is important to differentiate between the effective path length and the actual cell length. The

effective path length refers to the  $b$  in Lambert-Beer's Law  $A = abc$ , and is the mean distance transversed by the spectrophotometer light beam through the solution being measured. The actual cell length is the distance between the cell windows within the cell.

The effective path length of the instrument was measured and additional checks of linearity were made by comparing the absorbances of solutions obtained with the instrument to those read on a Cary 16 employing a cell of 10.0 cm actual cell length. In this experiment the assumption was made that for the 10 cm cell in the Cary 16 the actual cell length and effective path length are equal. The Cary 16 has been checked against established absorbance standards and the assumption shown to be correct.

Figure 12 shows the results obtained when solutions of methylene blue were measured at 650 nm in the microcell of 40 cm actual cell length (curve A) and the 10.0 cm cell of the Cary 16 (curve B). From the ratio of the slopes of these curves the effective path length is calculated to be 48.2 cm. Similar measurements were made with solutions of bromocresol purple, buffered at pH 10, at 590 nm; fast gray at 545 nm; red G at 520 nm, and glycinecresol red, buffered at pH 5, at 415 nm. All results showed good linearity and effective path lengths were in the range of 47 to 49 cm. The difference in the effective path length over the actual cell length can be understood if the internal reflection due to diverging light reflecting off of the cell walls is considered. Since the incident light is not parallel, it is to be expected that changes in the refractive index due to changing the solution within the cell would cause a different effective path length by altering the angles of the light rays striking

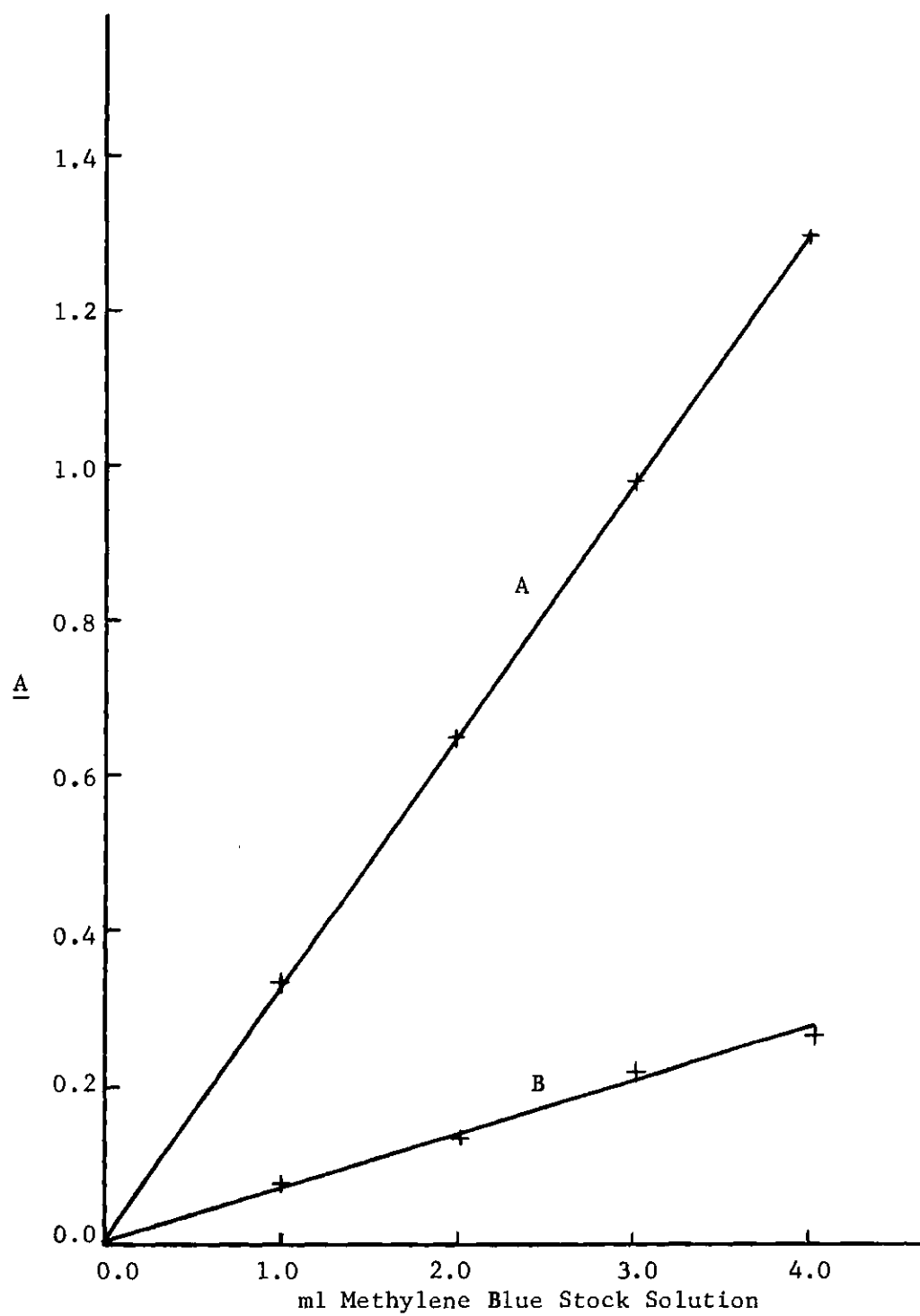


Figure 12. Measurement of Effective Path Length

the cell walls and thus increasing or decreasing the number of reflections occurring as the light passes down the tube.

To better understand the change in effective path length with the change in refractive index of the solution, the various interfaces transversed by the light beam in passing through the cell should be considered. It is obvious that no change in the angle of refraction occurs at either the air-glass interface of the cell window or the lens-air interface within the detector. Only the interfaces of the glass window with the solution and the solution with the detector lens should be considered when the refractive index of the solution is changed. When 100 percent transmittance is set with the appropriate solution blank, any variation in light reaching the light sensitive portion of the detector due to the angles of refraction at the later interface is compensated for by adjustment of the light intensity or by detector circuit adjustment. The interface of importance is thus the cell window-solution interface. Here, light not entering perpendicular to the window will be refracted. The greater the refractive index of the solution, the smaller the angle of refraction for a given ray and the shorter the effective path length. The smaller the refractive index of the solution, the greater the angle of refraction and the greater the effective path length.

A formula which can be derived for this relationship is

$$l' = \frac{l}{\sqrt{1 - \left(\frac{\sin \alpha}{n}\right)^2}} .$$

where  $\alpha$  is the angle of incidence to the interface,  $n$  is the refractive



index of the solution,  $l$  is the actual cell length, and  $l'$  is the effective path length. A derivation and figure explaining the origin of this formula are given in Appendix 3.

With the diverging light beam no definite value can be assigned to  $\alpha$ . However, for one specific positioning of the optical components,  $\alpha$  should remain constant and the equation can provide a general idea of the magnitude of the relationship. Experiments have shown the trend of increasing effective path length with decreasing refractive index as can be predicted from the formula. The order of magnitude of the changes observed in the effective path length also agrees with the formula.

Generally the refractive index of a substance increases with decreasing wavelength.<sup>38</sup> This variation constitutes about two percent of the refractive index between the spectral extremes in the visible range. Experiments have shown that this trend is also followed.

The fact that the spectral band width of the instrument (approximately 10 nm, constant) is about ten times that of the Cary 16 with normal settings may also cause a variation in the measured effective path length. However, experiments in which the spectral band width of the Cary 16 was increased by adjusting the slit have shown that this factor constitutes little of the observed variation when compounds with broad absorbance maxima are employed for measurement of the effective path length.

The effective path length may be appreciably varied by adjustments made within the optical path of the spectrophotometer.

The possible variation in effective path length, regardless of cause, is no problem at all if the general rule of photometric analysis

is followed, namely, to establish the calibration curve under conditions identical to those when measuring the sample.

## CHAPTER VIII

### EXAMPLES OF APPLICATION OF THE PHOTOMETER

The examples given in this chapter are intended to provide an indication of the instrument's potential for increasing the sensitivity of photometric determinations. The methods employed were not developed especially for the instrument, but rather are existing methods with only slight modifications in procedure and techniques for the low concentrations and small sample volumes which are applicable with the instrument.

The last two examples provide some ideas of the sensitivity increases obtainable by combining the techniques discussed in Chapters III and IV with photometric measurement in the long path microcells.

#### Photometric Determination of Manganese as Permanganate

##### by Standard Addition Technique

Manganese in high purity sodium carbonate was determined by means of a standard addition technique after being oxidized to permanganate with periodate according to the method of Cooper\*.<sup>39</sup> A solution of 5.77 mg potassium permanganate per 100 ml of saturated potassium periodate solution was used as a stock solution and added in 4 $\lambda$  increments for the standard addition. The 2 ml sample (in acidic periodate solution) was

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\*The high purity sodium carbonate sample solution was provided by courtesy of J. T. Baker Chemical Company.

introduced into the cell reservoir and cell, and the stock solution added by means of a micropipette. After each addition of stock solution the sample was mixed by alternately pushing the sample into the reservoir and pulling it into the cell with several strokes of the syringe. The absorbance readings were made at 545 nm.

Figure 13 shows a plot of the experimental data. No volume corrections were made since the volume change was negligible. Best results were obtained by "preoxidizing" the long-path microcell and reservoir with dilute permanganate solution and flushing with distilled-deionized water.

#### Photometric Titration of Copper(II) with EDTA

##### Using PAN as an Indicator

Employing the long path spectrophotometer as a phototitrator, copper(II) was titrated with DETA using PAN as the indicator. The titration was performed in the 2 ml reservoir attached to the larger (approximately 0.8 ml capacity) microcell. To 1.50 ml of  $1.0 \times 10^{-7}$  M copper (II) solution was added 0.1 ml of pH 5 acetate buffer, 0.5 ml of 95 percent ethanol, and a small drop of  $10^{-5}$  F PAN solution. The resulting solution was titrated with  $1.0 \times 10^{-5}$  F EDTA solution dispensed from a microburet and the absorbance changes at 560 nm recorded. After each addition of titrant homogeneity was obtained by pulling solution into the cell and pushing it back into the reservoir several times with the syringe.

Figure 14 shows the plot of the titration data. No correction has been made for dilution since the titrant is much more concentrated than the solution being titrated. For this titration 100 percent transmittance was set with the buffered sample solution prior to addition of the PAN indicator.

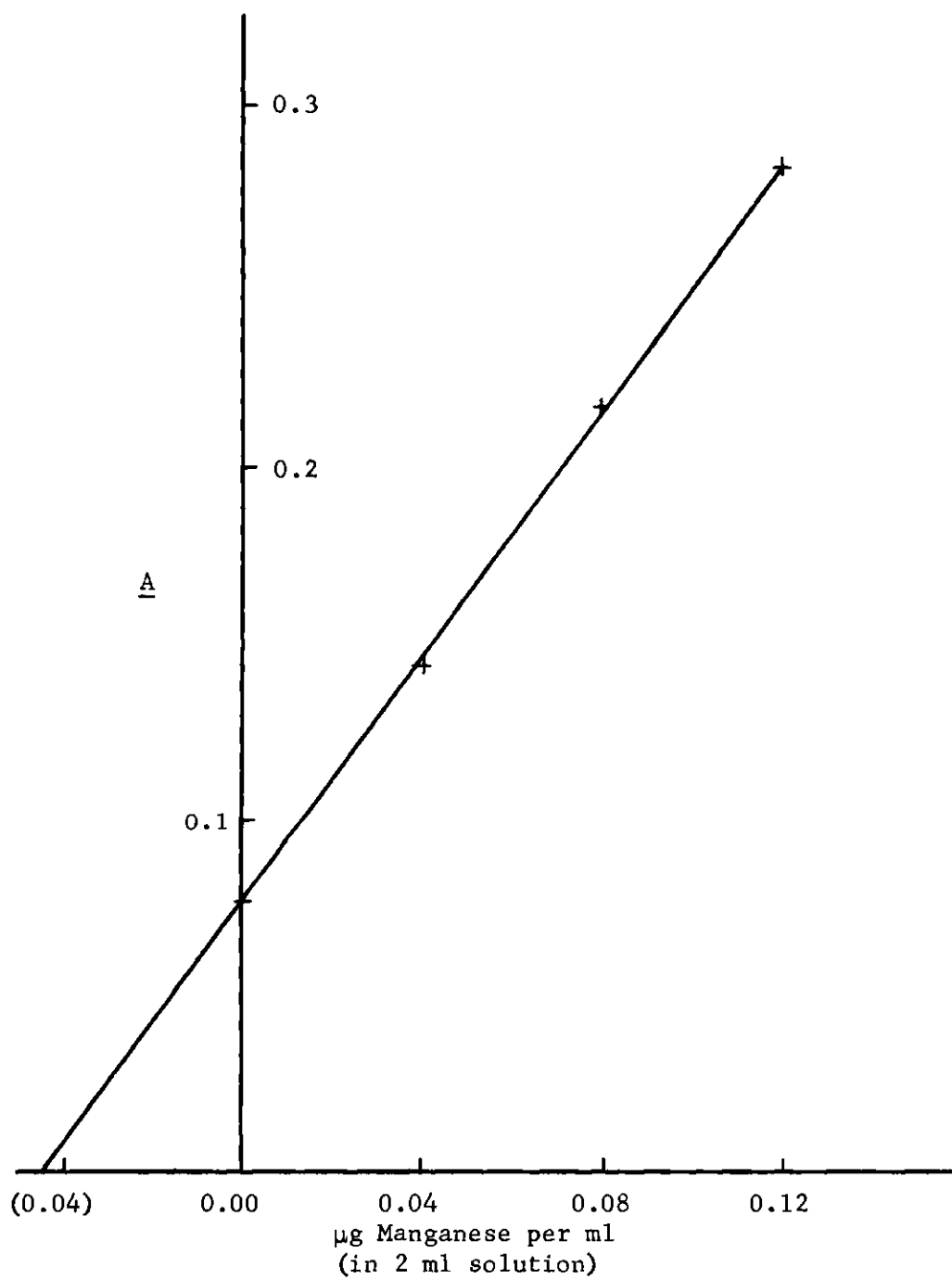


Figure 13. Photometric Determination of Manganese as Permanganate

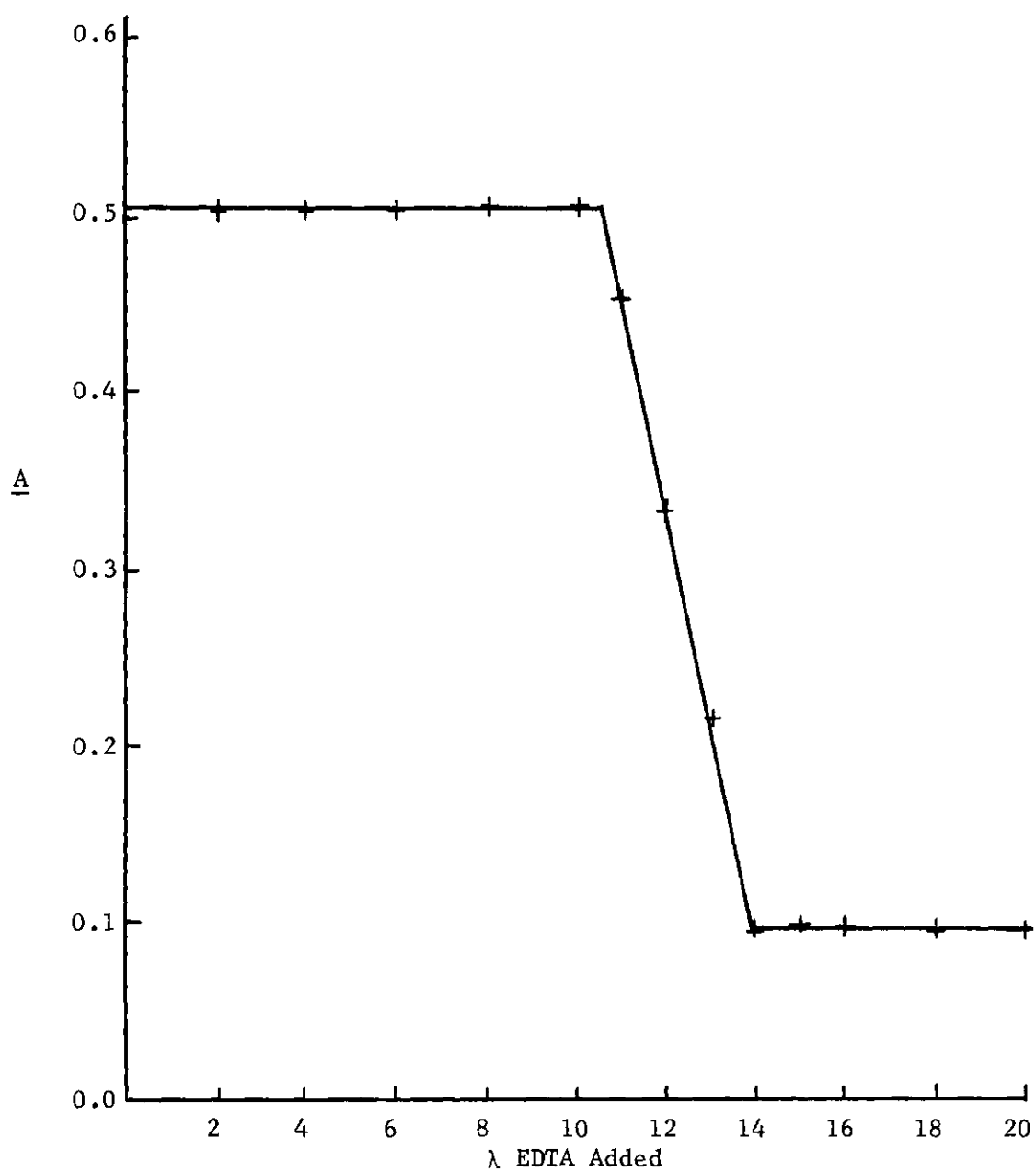


Figure 14. Photometric Titration of Copper(II) with EDTA

Photometric Determination of Manganese with PAN Utilizing  
the Solid Homogeneous Extraction Technique

Manganese in high purity sodium carbonate\* was determined with PAN according to the method of Donaldson<sup>40</sup> using appropriate volume modifications and employing the "solid-homogeneous extraction" technique for extraction of the complex. To a 2 ml aqueous sample containing 0.01 to about 0.08  $\mu\text{g}$  of manganese(II) was added 0.1 ml of 10 percent sodium tartrate solution, and 0.4 ml of 20 percent hydroxylamine hydrochloride. The solution was mixed, 0.4 ml buffer (10 g of ammonium chloride dissolved in water, 100 ml of concentrated aqueous ammonia and 1.2 g of potassium cyanide added, and diluted to 200 ml with water), 0.05 ml 0.1 percent PAN in ethanol added, and the solution again mixed. The complex was then extracted into 0.4 g of piperonal by the solid-homogeneous extraction technique as described in Chapter III. The solid solvent bead was dissolved in 3 g of m-xylene and the absorbance measured at 560 nm in the long-path microcell.

Figure 15 shows a calibration curve for this method. A blank was prepared by using 2 ml of oxidizing solution for the sample and 100 percent transmittance was set with this blank. No attempt was made to achieve high enrichment prior to the photometric determination since with the described procedure, the sample of interest gave an absorbance reading greater than 1.0

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\*See footnote page 79.

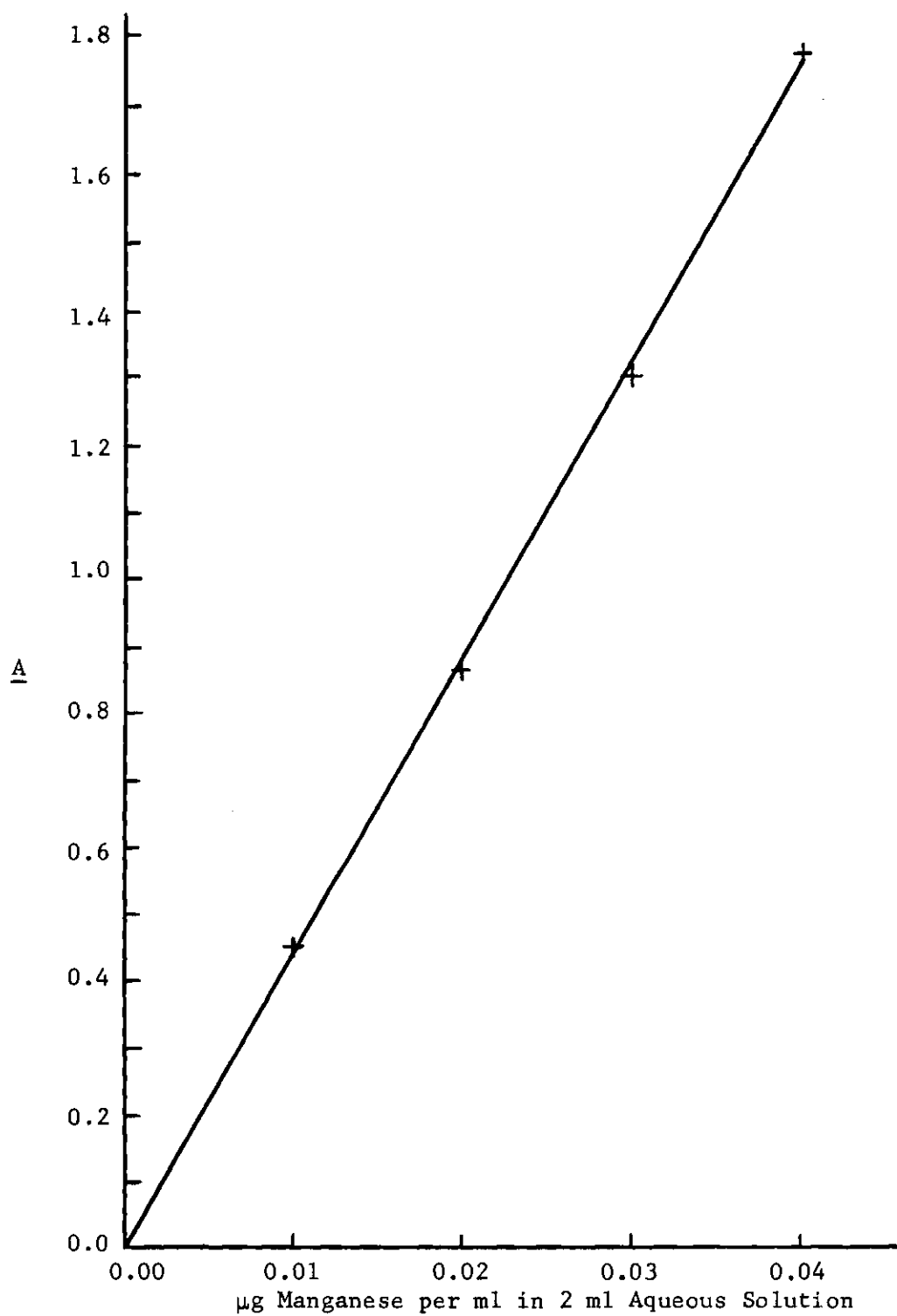


Figure 15. Photometric Determination of Manganese with PAN



Photometric Determination of Cobalt (II) as the Thiocyanate Complex  
with Sulfolane as an Enhancing Agent

This example combines the sensitivity increase due to the long-path cell with the sensitivity increase from a higher molar absorptivity. Sulfolane provides two to three times greater absorptivity of the complex than the best organic enhancing solvents previously employed. With the effective path length of the microcell about 48 cm, the combination should provide a sensitivity increase of more than two orders of magnitude.

Figure 16 shows a calibration curve established according to the following procedure. To a 10 ml volumetric flask is added 5 ml of sulfolane, 2 ml of 2.5 M potassium thiocyanate, and from 0 to 0.5 ml of  $1.0 \times 10^{-4}$  M cobalt(II). The solution is diluted to volume and the absorbance measured at 620 nm. Sensitivity is, as expected, over 100 times greater than usual methods based on the Vogel reaction<sup>23,24,25</sup> and the determination of as little as 0.06  $\mu\text{g}$  of cobalt is possible with this method.

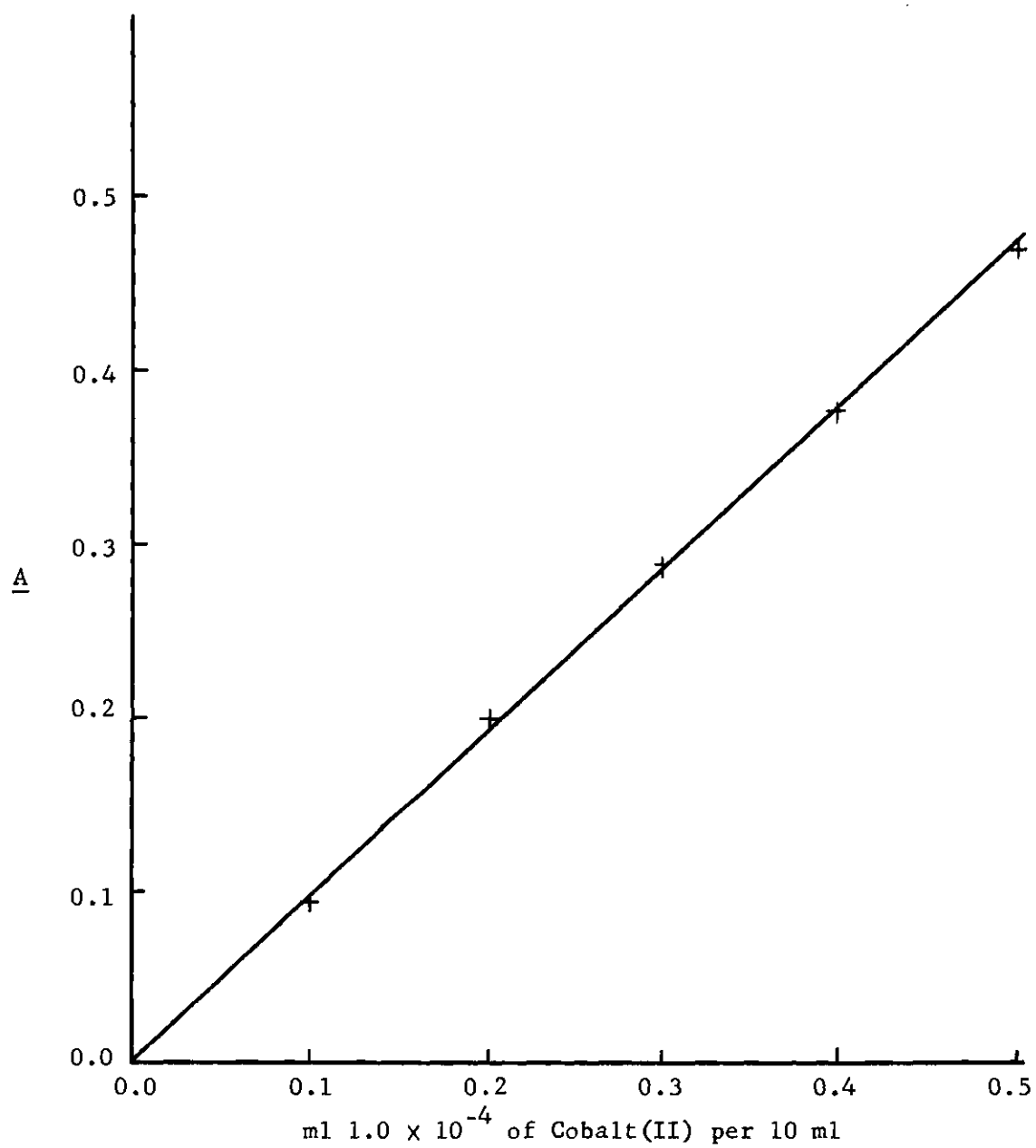


Figure 16. Photometric Determination of Cobalt as the Thiocyanate Complex

## APPENDIX I

## INTERNAL CALIBRATION OF THE PHOTODIODE CIRCUIT

## A. To Zero Amplifiers A1, A2, and A3:

1. Place the mode switch in the "%T" position.
2. Disconnect the photodiode from the circuit.
3. Connect a clip lead from the wiper of the "set 0% T" potentiometer to ground.
4. Connect a digital voltmeter to the output of A1 and adjust the corresponding 100 K trimpot for a reading of  $0 \pm 0.5$  mV on the DVM.
5. Connect the DVM to the output of A2 and adjust the corresponding 10 K trimpot for a reading of  $0 \pm 0.1$  mV on the DVM.
6. Connect the DVM to the output of A3 and adjust the corresponding 10 K trimpot for a reading of  $0 \pm 0.5$  mV on the DVM.

## B. To Calibrate the Voltage Offset:

1. Remove the clip lead indicated in step A.
2. Substitute a 20 m resistor for the photodiode.
3. Adjust the "100% T set" control to obtain a reading of 1.000 V at the A3 (normal) output.
4. Switch to the "% T X 2" position and set the voltage offset control to 100 percent (ten turns).
5. Adjust the "offset calibrate control" (50 K trimpot) for 1.000 V at the output.

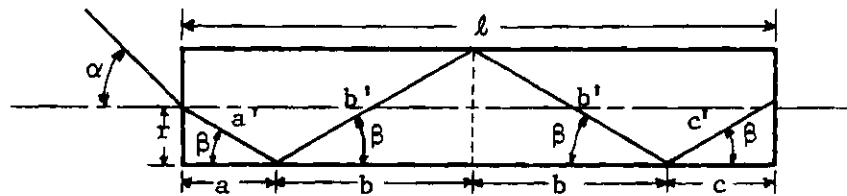
C. To Set Zero "log T":

1. Set a 100 percent transmittance reading of 1.000 V as in step B.
2. Adjust the "set zero log T" control (1 M trimpot) to obtain a 0.000 V reading.
3. More precise adjustment of the "log T" mode may be obtained when the circuit is attached to the photodiode under actual operating conditions.

## APPENDIX II

## DERIVATION OF THE FORMULA

$$l' = \frac{l}{\sqrt{1 - \left(\frac{\sin \alpha}{n}\right)^2}}$$



$$l = a + mb + c$$

$$l' = a' + mb' + c'$$

$$\tan \beta = \frac{r}{a} = \frac{2r}{b}$$

$$\cos \beta = \frac{a}{a'} = \frac{b}{b'} = \frac{c}{c'}$$

$$a' = \frac{a}{\cos \beta} \quad b' = \frac{b}{\cos \beta} \quad c' = \frac{c}{\cos \beta}$$

$$l' = \frac{a}{\cos \beta} + \frac{mb}{\cos \beta} + \frac{c}{\cos \beta}$$

$$l' = \frac{1}{\cos \beta} [a + mb + (l - a - mb)]$$

$$\frac{\sin \alpha}{\sin \beta} = n$$

$$\sin \beta = \frac{\sin \alpha}{n}$$

$$l' = \frac{l}{\cos \beta}$$

$$\cos \beta = \sqrt{1 - (\sin \beta)^2} = \sqrt{1 - \frac{(\sin \alpha)^2}{n^2}}$$

$$l' = \frac{l}{\cos \beta} = \frac{l}{\sqrt{1 - \left(\frac{\sin \alpha}{n}\right)^2}}$$

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## VITA

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In July, 1970, he was married to Janet Ruth Drennan of Elberton, Georgia.